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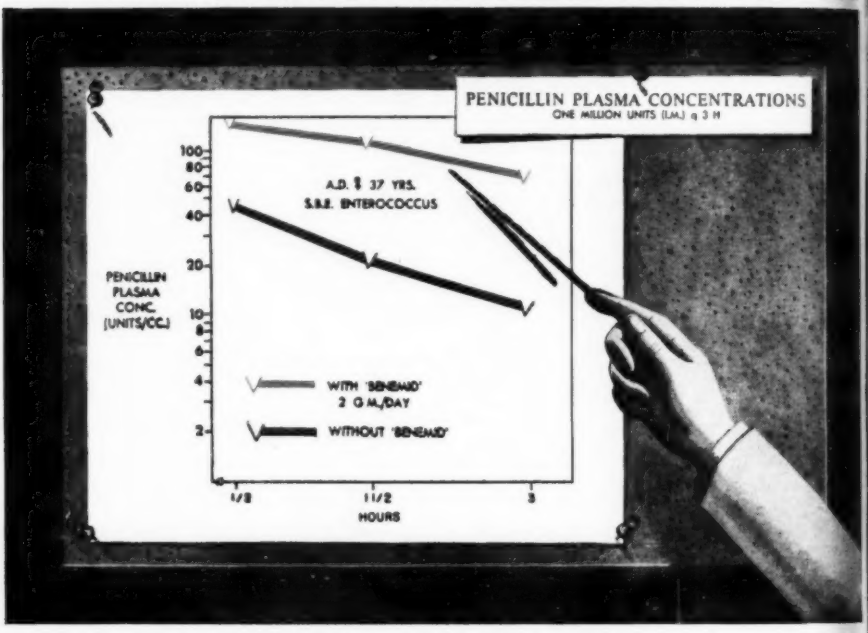

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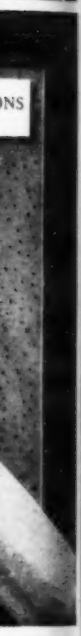

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THE DIFFERENTIAL EFFECT OF MORPHINE ON CARDIAC AND MOTOR CONDITIONAL REFLEXES—SCHIZOKINESIS[1]

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Received for publication November 23, 1955

INTRODUCTION

A useful index of cerebral function is the conditional reflex. Both its general visceral components, e.g., cardiac, and its specific components, e.g., movements and secretion, can be quantitatively recorded (3, 4, 5, 6, 10, 15, 19). The action of morphine is well known as a depressant of pain. The analysis of this action by the study of its effect on general behavior, on the cardiac, respiratory, and motor components show that morphine has a differential effect on the internal, visceral and on the external, motor components of acquired behavior, but much less effect on the inborn unconditional responses (both motor and cardiac) to pain. The results to be presented are further evidence of the *great stability of the acquired cardiac responses*, the difficulty of removing them once they are established even when a powerful analgesic is used. This split in function between the stable cardiac conditional responses and the motor conditional reflex is what has been previously referred to in our laboratory as schizokinesis (7, 13).

METHOD

Five dogs, Crazy, Tichie, Skipper, Chow and Rocky were used in these experiments. Each, having been trained in the laboratory for several years, had well established conditional reflexes to faradic shock, with reliable and ample records of the cardiac, respiratory and motor components. As their reactivity had been measured to other drugs and procedures, as will be mentioned, these past records give a standard of comparison. The experiments were conducted in a sound-proof Pavlovian "camera". On each experimental day, the dog was placed in this room where two tones an octave apart were sounded in alternation every two minutes. The lower pitched tone (T256+) was the positive (excitatory) conditional stimulus always immediately followed by the unconditional stimulus, a faradic shock of one second duration to the left front paw of the dog. The higher pitched tone (T512−) was the negative (inhibitory) conditional stimulus not followed by a shock. These two tones were given alternately for 5 seconds each at exactly two minute intervals. In order to obtain constant conditions of reenforcement, shock inevitably succeeded T256, the dog not being able to avoid the shock by lifting his paw. This procedure was repeated several hundred times until a stable conditional reflex had been established, i.e. the heart rate changed regularly and the dog moved his foot when the positive conditional stimulus was sounded. Kymographic readings were made of the leg movement, and an electrocardiogram was taken to measure the heart rate. Respiration was pneumatically recorded. One dog, Tichie, was given only the positive (reenforced) stimulus. During the present series of experiments on alter-

[1] Aided by a Grant from the American Heart Association.

nate experimental days the dogs were given morphine in doses of from 0.5 to 5.5 mg./kg. subcutaneously, and after sixty minutes the dogs were placed in the experimental room and recordings made of the conditional reflexes. On the other experimental days, the same procedure was done without the drug.

Description of dogs

Crazy: Male dog, born about 1943. This dog is hyperreactive, indifferent toward people, asocial, makes circus movements to left, has increased tone and reflexes on right, with impairment of the placing and hopping reactions. Hemi-decortication left side and extirpation gyrus cingulus were performed on right side about 1946. Positive and negative conditional reflexes were established to T256+ and T512−. Upwards of 2000 repetitions were given to this dog before morphine experiments were begun. Good motor and cardiac differentiation was obtained with a somewhat impaired time reflex (8) (11).

Tichie: Male, hound type, born about 1942. Very phlegmatic, friendly, though does not pay much attention to experimenter; slow in movements. We began laboratory work with him about 1948. Only T256+ was given, without any inhibitory conditional stimulus. Forms conditional reflexes quickly, the cardiac conditional reflex on third trial, the motor conditional reflex about the 10th. When morphine experiments were begun 200 repetitions of T256+ reenforced with shock had been given (9).

Skipper: Female, born about 1946, very friendly and affectionate, playful, sanguine type, active. Experimentation began in 1950 with T256+ and T512−. Excellent differentiation, both motor and cardiac conditional reflexes, with good cardiac time reflex to the 2 minute regular intervals of the alternation of T256+ and T512−. Over 900 repetitions of each conditional stimulus had been given before morphine experiments were started in 1952 (12).

RESULTS

A. Without morphine (Control)

Skipper and Tichie showed very constant responses to the conditional stimuli. During the two week period before morphine was first administered, both dogs gave a positive motor response (withdrawal of the paw) to the excitatory stimulus for 100 per cent of the trials. There was no motor response to the inhibitory tone (0 per cent). Cardiac rate increased to both excitatory and inhibitory stimuli but to a lesser degree to the inhibitory tone. Respiration changed in amplitude or frequency to the conditional stimuli 100 per cent of the time when measured.

Crazy, having been less well trained, during this two week period, gave a positive motor response to the excitatory conditional stimuli from 60 to 100 per cent of the time, with an average of 73 per cent. There was no motor response to the inhibitory tone. Heart rate increased to the excitatory tone and decreased to the inhibitory tone. Respiration was not recorded in Crazy during morphine.

B. With morphine

The data obtained when morphine was given will be presented under four headings, 1) activity, 2) motor conditional response, 3) cardiac conditional response, 4) respiratory conditional response.

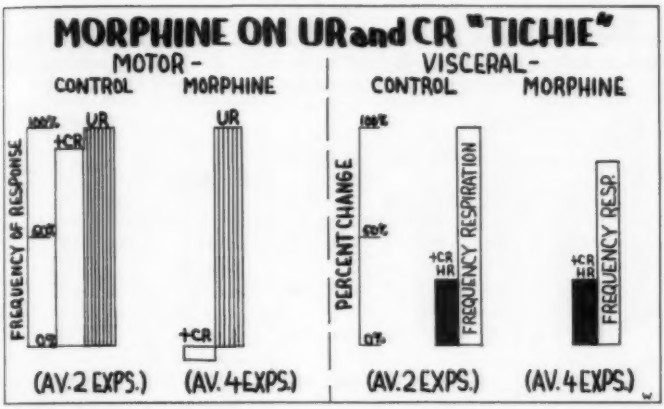

FIG. 1. On the left (motor) is shown the effect of morphine in abolishing the motor CRs without affecting the motor URs. On the right (visceral), is shown the lack of effect of morphine on the *reactivity* of the heart to the signals for painful stimuli. The absolute HR is markedly lowered by morphine, but the chart shows only per cent changes.

1) *Activity:* Tichie was given four doses of morphine, 5, 2, 1 and 0.5 mgs. per kg. With the largest dose he became lethargic: he would not walk, having to be carried into the experimental room. The intermediate doses caused a slightly ataxic gait, while the lowest dose did not show a change in behavior. The dog was very quiet during the experiment with all doses, sometimes lying down.

Skipper was given three doses of morphine, 3.2, 1 and 5 mgs. per kg. With the dose of 3.2 mgs. per kg., which was given first, the effect was greater than with subsequent larger doses; the dog would not walk, had to be carried into the camera, and was very lethargic throughout the experiment. The dose of 1 mg. per kg. had no demonstrable effect; there was no ataxia—the dog jumped onto the platform in the camera. She was very active throughout the experiment, frequently barking and struggling. A dose of 5 mgs. per kg. produced a slightly ataxic gait and some lethargy.

Crazy was given three doses of morphine, 2, 1 and 0.5 mg. per kg. With the two larger doses the dog would not walk, had to be carried into the camera, where he remained in a semi-recumbent position through the experiment. Even the 0.5 mg. dose produced an ataxic gait and lethargy.

Morphine did not cause sleep in any of the dogs in any dose, nor alter deep tendon and placing reflexes.

2) *Motor conditional response:* In Tichie morphine in all doses completely *abolished* the motor conditional response. The motor unconditional responses were not significantly altered. The amplitude of the response to the shock was not changed by morphine (See Fig. 1 and Table I).

In Crazy morphine in all doses also abolished the positive motor conditional

TABLE I

Effect of Morphine in Two Dogs on 1) Motor Excitatory Conditional Reflex (+CR), Inhibitory Conditional Reflex (−CR), Unconditional Reflex (UR), 2) Cardiac Conditional Reflex (HR.− CR), Respiratory Conditional Reflex Shown as Per Cent of Control*

"Skipper"								"Tichie"				
Exp. & Date	Motor			HR-CR% inc.		Respiratory CR		Exp. & Date	Motor		HR-CR % inc.	Resp. CR
	+CR	−CR	UR	+CR	−CR	+	−		+CR	UR		
Control, May 12	100	0	100	60	5	100	100	Control, May 12	100	100	22	100
3.2 mg/kg, May 13	*60*	*0*	*100*	*52*	*−3*	100	100	5 mg/kg, May 13	*0*	*100*	*27*	*30*
1 mg/kg, May 16	*100*	*0*	*100*	*50*	*6*	100	100	2 mg/kg, May 16	*0*	*80*	*33*	*100*
5 mg/kg, May 22	*80*	*20*	*90*	*35*	*2*	100	100	1 mg/kg, May 26	*0*	*90*	*24*	*100*
Control, June 16	100	0	100	44	9	100	100	Control, May 28	90	100	20	100
5.5 mg/kg., June 18 1952	*60*	*0*	*100*	*41*	*−3*	100	100	Control, June 13	100	100	34	100
								0.5 mg/kg, June 17	*0*	*100*	*24*	*100*
Average												
Control	100	0	100	52	7	100	100	Control	97	100	25	100
Morphine	*75*	*5*	*98*	*45*	*2*	*100*	*100*	Morphine	*0*	*92*	*27*	*82*

* Morphine readings in italics

response. The negative motor conditional response was unchanged. The motor unconditional response was not altered.

In Skipper the 1 mg. per kg. dose had no effect on any of the motor conditional responses, and the dog maintained his ordinary activity during the experiment. The 3.2 mg. per kg. dose decreased but did not abolish the positive motor conditional response; the negative motor conditional response was unaltered. The 5 mg. per kg. dose also decreased but did not abolish the positive motor conditional response; there was, however, further loss of differentiation in that there was a 20 per cent instead of a zero response to the inhibitory tone. Motor unconditional responses were not significantly changed by any of these doses (Fig. 2 and Table I).

3) *Cardiac conditional response:* In Tichie morphine in all doses given caused cardiac irregularities and *reduced the rate* to one half to one third of the resting control values. Morphine, however, *did not abolish* the cardiac conditional response—the percentage increase of heart rate to the conditional stimulus was not significantly altered by the drug. (Fig. 1).

In Crazy as the heart rate was slow and very irregular even without morphine, the results were not very significant. The morphine *did not however*

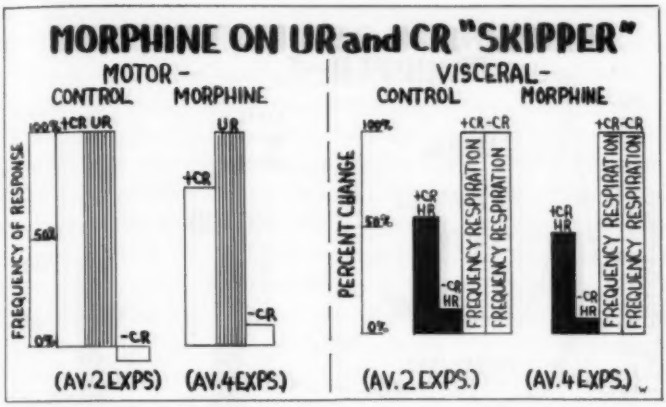

FIG. 2. On the left is shown the reduction of excitatory motor CRs without effect on the inhibitory motor CRs or on the URs. On the right (visceral) there is practically no effect on the cardiac *reactivity* to signals for pain, either excitatory or inhibitory.

abolish the cardiac conditional response although it in general seemed to decrease the percentage change.

In Skipper morphine in all doses given caused cardiac irregularities and reduced the rate. It *did not*, however, markedly alter the percentage change in rate to the excitatory and inhibitory tones (Figs. 2 and 3).

One of the dogs, Skipper, showed a marked cardiac reflex to time intervals as previously reported by this laboratory (11). This was abolished completely by morphine.

4) *Respiratory conditional response:* In Tichie only the largest dose of morphine, that of 5 mg. per kg. depressed the respiratory conditional responses (Fig. 1).

In Skipper the respiratory conditional response was not altered by morphine (Fig. 2).

In Crazy technical difficulties prevented recording of respiration in an adequate number of controls for comparison.

Rocky, a dog used previously for the study of mescaline, was given 4.6 mg./kg. morphine: the motor crs were reduced from 100 per cent control to 20 per cent with morphine, but the cardiac positive crs were increased from a control of 20 per cent differentiation in heart rate to 42 per cent differentiation under morphine (experiments of Wagner Bridger).

Mescaline, a drug known to alter subjective states in the human being, was tried on three of the same dogs (Skipper, Chow, Rocky) which were used in the morphine experiments. Its effect on the motor and cardiac conditional reflexes was almost diametrically opposite to that of morphine: in doses of .35

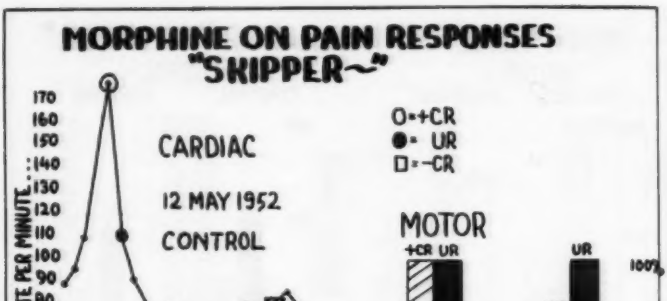

FIG. 3. On the left is shown the cardiac reactivity, control and under morphine, to signals for painful stimuli and to the painful stimuli. Though the HR level is markedly reduced by morphine, the *reactivity* in per cent is unaltered. On the right is shown the reduction of the motor CRs but not the motor URs by morphine.

mg./kg. the motor conditional reflexes were reduced from 100 per cent control to 90 per cent in Chow and Rocky and to 80 per cent in Skipper, while the cardiac conditional reflexes were reduced from 28 per cent increase to 2 per cent in Skipper, from 28 per cent increase control to 3 per cent in Rocky, from 21 per cent increase control to 6 per cent in Butch, and from 58 per cent increase control to −2 per cent in Skipper (2).

DISCUSSION

Two aspects of the action of morphine relative to conditioning have been investigated—its use as an unconditional stimulus, and its effect on other formed conditional reflexes. As an example of the first use, Krylov (20) of the Pavlov School showed that morphine could function as an unconditional stimulus; salivation, vomiting and sleep could be conditioned in dogs to the preliminaries of the injection of morphine. Secondly, morphine in cats was shown to abolish the motor conditional reflexes (17). In decorticate dogs and cats, Wikler (22) showed that morphine abolished or reduced sham rage responses, as well as "autonomic responses".

No measurement has been made of the cardiac component of the conditional reflex previous to our experiments.

The action of morphine on pain reflexes, like the results of many experi-

ments, was contrary to our anticipation and hypothesis, viz. that it would have a greater effect on the responses to the actual pain (unconditional reflex) than on the responses to the signals for pain (conditional reflexes), and especially that its action would be more marked on the acquired cardiac responses (emotional factor?) than on the acquired motor responses. The reverse effects, however, appeared, the most marked being on the "voluntary" motor conditional reflexes, the per cent effect on the visceral conditional reflex (HR) being negligible. Since morphine is known to reduce pain primarily,[2] and the emotional feelings based thereon, we definitely expected that the effect on the acquired visceral responses (generally considered as chief components of emotion) would predominate over the effect on the motor response, and that there would be an abolition of the cardiac conditional reflexes. The action of morphine, as we know it subjectively in the human, is to allay the feeling of pain and to make us less reactive to pain. The effect on the dog is apparently similar in its observed effect on the human. How then can we reconcile its subjective action with the almost insignificant effect on the pain unconditional reflexes, and no effect on the cardiac component of the pain conditional reflex (as measured by the per cent change from the resting level of heart rate) and yet a more pronounced effect on the less emotional component (motor)?[3]

A partial and hypothetical answer may be given. Pain is a complex function, involving both acquired anticipations to its signals, i.e. conditional reflexes as well as inborn unconditional reflexes. The evidence for this, though commonplace, is not often analyzed.

Confusion results because pain is sometimes considered from the subjective aspect alone and sometimes only objectively. The fact that morphine does not eliminate the learned cardiac component of behavior though it reduces the accompanying *feeling* of pain brings up the question as to the significance and interpretation of the cardiac conditional reflex which we measure. In spite of the established view that the visceral reactions, especially the cardiorespiratory, are at the basis of the subjective feeling of pain, as has been stated in the James-Lange Theory and implied by Cannon and others, this *cardiac component may not be the essence of the emotion nor of the subjective feeling* of pain. Whitehorn (21) has shown that many psychiatric patients do not show acceleration of cardiac rate even under emotional stress.

[2] Wolff and collaborators state that morphine raises the threshold to pain stimuli, produces freedom from anxiety, relaxation, apathy, feelings of contentment. "While in these states the subjects perceived pain, but the usual reaction pattern to pain was altered. . . . An opiate accentuates the ability to dissociate pain perception from the pattern of pain reaction". (23).

[3] The marked reduction in heart rate level calls for evaluation also. This does not seem as important to us in the discussion as the fact of the retention under morphine of the percent difference between the resting HR and the increased HR during the action of the conditional signal.

Schizokinesis has been described by one of us (13, 14) as a splitting between the more voluntary specific external aspects of the conditional reflex, e.g. the somatic motor responses, and the more involuntary, general, internal, visceral components of the conditional reflex, viz. heart rate and respiration. The somatic motor aspect is usually the last to form and the first to disappear so that the individual may show inhibition of the motor conditional reflex but retain the visceral. The action of morphine primarily on the specific motor conditional reflex and its failure to affect the visceral component of the conditional reflex is another example of the phenomenon of schizokinesis.

Schizokinesis in most of the cases we have studied applies to the preservation of the cardiorespiratory responses in the presence of the inhibition of the somatic motor responses, but we (Bridger and Gantt) have seen at least one example of a split in the other direction, viz. in the action of mescaline, which abolishes the cardiac conditional reflex before it does the motor (2). Mescaline however is a drug producing abnormal reactions that are poorly understood, and its action may come in another category.

Mowrer in discussing learning recognized the difference between the somatic and the visceral elements under the term "dual factor of learning" (18). This split function has been emphasized by one of us (13) as a basis for an inborn lack of adaptation to a changing environment and perhaps as a cause of psychiatric disorders.

Additional evidence of schizokinesis has been produced by the ingenious experiments of Paul MacLean (16). He showed that after an electrical stimulus of the gyrus cingulus, motor conditioning but not cardiac, disappeared. In a dog with a subconvulsive shock through electrodes implanted in the hippocampus, in this laboratory we have shown that the motor conditional reflex disappears for some hours though the cardiac is unaffected (1).

It is possible in applying these results obtained in animal experiments to human behavior to draw an analogy to the observation that the somatic responses accompanying stressful situations may be absent in contrast to the marked visceral changes occurring. It is of interest to note here that the hypertensive patient very frequently appears outwardly calm and unmoved by a stressful situation when at the same time his blood pressure and heart rate are varying markedly.

Even a powerful drug like morphine, which can abolish the feeling of pain and the motor conditional reflexes to the signals to pain, and can lower the level of the heart rate in our dogs to one-half its normal value, nevertheless fails to reduce the percentage rise in the cardiac conditional reflex.

We are grateful to Dr. A. Wikler, who has made extensive studies of the action of morphine, for suggesting to us the advantage of using morphine in the investigation of the cardiac component of the conditional reflex.

SUMMARY

Morphine in doses sufficient to produce lethargy and ataxia *abolished or reduced the somatic motor conditional response but not the somatic unconditional response*. In contrast to the effect on the motor learned behavior, the *cardiac* component of the pain conditional response in terms of percentage change in heart rate was hardly changed, though the resting level of rate was markedly lowered by morphine.

This work confirms our observations that the cardiac conditional response in dogs is 1) an important component of acquired behavior, 2) usually more persistent than the motor conditional response, and 3) there is a split or *schizokinesis* between the external motor and internal visceral functions.

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ELECTRON MICROSCOPIC EVIDENCE CONCERNING THE MAMMARY TUMOR INCITER (VIRUS)

I. A Study of Normal and Malignant Cells from the Mammary Gland of Mice

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Received for publication November 30, 1955

Several comparative studies of normal and malignant cells by electron microscopy have failed to establish significant differences in ultrastructure (1, 2, 3). As techniques of preparation of cells (4, 5) continue to improve, however, further comparison is indicated. Two limitations in present techniques should be recognized. First, the samples of tissue prepared for the electron microscope are so small that differences may simply be missed in the samples chosen for study. Second, it must be emphasized that identifiable virus particles can be found in the electron microscope only if they are present in large numbers (6). Porter and Thompson reported in 1948 that virus-like particles were found in several mammary tumors prepared for electron microscopy by tissue culture techniques. Since then preliminary notes on thin sections of mammary tumors in mice have been published or presented by several groups (8, 9, 10, 11).

Characteristic large numbers of virus-like particles have been found in the tumor cells and within the altered microvilli which extend from them (9, 10). The tumor cells are usually not destroyed in the presence of these particles, which differ from the normal components of most secreting tissues in that they are uniform in size, have dense centers and do not present the sharp outline that casein or protein droplets do. A number of the tumors have shown clusters of particles around what seem to be altered mitochondria. Scattered individual particles are present within the mitochondria. They occur in cells which are not apparently secreting milk. It is the purpose of this paper to present our findings on six spontaneous mammary tumors from C_3H mice known to carry the inciter of mammary tumors (MTI). These are compared with normal mammary tissue from virgin and lactating mice with and without the inciter.

MATERIALS AND METHODS

This study was started in 1951 and some of the methods of preparation have changed during the study. We will indicate differences in techniques in the text where they seem to have influenced the results. All tumors were obtained from mice which had been killed immediately beforehand. Of the six tumors, five occurred spontaneously within the C_3H line of mice obtained from Dr. H. B. Andervont in October 1951 and kept as a colony at

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Johns Hopkins since then. The earliest was at six months of age. One (#743) occurred in an inciter-free C_3H mouse from a colony which had been freed from the inciter by Dr. Andervont by cesarian section and foster nursing. This mouse had, however, been injected by us with the inciter shortly after birth and developed its tumor at 15 months of age. In several of the tumors sections were cut from two different places. Sections were made from mammary glands of five virgin mice, and from lactating mammary glands of nine mice. Three of these latter had the inciter.

In all cases fixation was with 1 per cent or 2 per cent buffered osmium, and was done by cutting tiny pieces in the osmium tetroxide itself. Tissues were fixed for four hours before being transferred into water. They were dehydrated in a series of ethyl alcohol solutions. They were embedded in a mixture of four parts of butyl and one part of methyl methacrylate. The original temperature [illegible]olymerization was 45°C. However, Borysko (13) has shown that fewer artifacts a[illegible]ced at higher temperatures. For this reason during the last year all embeddings [illegible]en polymerized at 60°C. Initially sections were cut by a modified Spencer microtome and mounted in collodion after the methacrylate was removed. The sections currently are cut with a Porter-Blum microtome (15) and are mounted on a collodion film without removing the methacrylate. The electron microscope is an EMU-RCA 50 KV microscope.

RESULTS

Since most of our efforts have been on tumor cells, the general characteristics of these cells will be described first. As shown in Figure 1 most of the cells in the tissues we studied were fairly dense and compactly arranged in acini. They did not differ significantly from the descriptions of Dunn for Type A tumors (16). When capillaries were present in a section the endothelial cells seemed to be separated from the tumor itself by a layer of diffuse and poorly organized material. An occasional cell below the endothelium appeared to differ from the tumor itself. The surface of the endothelium of the capillaries was usually normal in appearance although the mitochondria of these cells were often ballooned. Particles resembling virus were not seen in the endothelial cells. The tumor cells were closely packed together and had blunt club-shaped processes which extended from one cell into the other so that the surfaces of the two cells often seemed interlocked (Fig. 2). There were often condensations of the cell membrane at opposing surfaces. We have called these complementary granules (17). In general the cytoplasm of neighboring cells seemed to be separated. However, at some places the destruction produced either during the process of polymerization or by the cutting was such that it was difficult to follow the cell membranes continuously and the impression of interconnection of the cytoplasm of two cells might be suggested. We do not think that this conclusion is justifiable with present evidence. Mitochondria usually had their characteristic shape and contained the familiar internal structure (18, 19). The cytoplasm had relatively little endoplasmic reticulum and in many places the tiny particles which are tentatively identified as ribonucleic acid by Palade and Siekovitz (20) occurred in great numbers where reticulum was not present at all.

The nuclei were separated from the cytoplasm by the usual space so that an apparent double nuclear membrane was observed (21, 22). However, the distance between the two parts of this membrane varied from place to place and the findings could fit the idea of a separate nucleus with its individual membrane within the cytoplasm which also has its membrane (Fig. 3). The "double" nuclear membrane thus seemed identical in appearance to the double appearance of two adjoining cytoplasmic membranes. The nuclei were roughly spherical with few indentations. Tiny dots were seen throughout and in greater concentration towards the surface of the nucleus (Figs. 2, 3). The nucleoli usually lacked the compact filamentous form which has been found in a variety of other types of cells (23, 24, 25). In most cases the fine structure of these nucleoli consisted of an increased concentration of the same fine particles seen in the rest of the nuclei and there was no sharp difference from the rest of the nucleus. In one of the tumors a mitotic figure was found and studied in serial sections. Again the ultrastructure of these chromosomes did not seem to differ from the nucleoplasm itself and consisted of fine individual particles. Some of these seemed to be linear and the entire basic structure may represent very fine filaments which have been cut in cross section and diagonally (Figs. 4, 5, 6).

The most interesting findings in these tumors related to certain virus-like particles. When they were found buried in the cytoplasm they occurred as small, empty looking spheres of about 70 mμ in diameter. When in clusters at the surface they measured 130 mμ and had dense centers corresponding to the smaller sized particle. They were first noticed in the relatively thick sections cut with the Spencer microtome, and in these kept their essentially round shape. They were often found in small vesicles which could be either pockets of fluid within the cell or pockets of fluid between cells (Figs. 7, 8). Projecting into these fluid vesicles were microvilli (Figs. 9, 10) which differed from the normal microvilli in two respects; 1) they were frequently irregular in thickness and were branched, and 2) they had within them dense particles which usually occurred at the tips. These dense particles were either eccentric or central and occupied about one-half the diameter of the microvilli. Serial sections were made through one of these vesicles in which the microvilli were few and short and a reconstruction was drawn from a series of tracings. It showed that the particles occurred in clusters at the surface of the cell and were apparently then extruded at different points (Figs. 11, 12).

A characteristic location for the smaller particles was within the cytoplasm (Figs. 13, 14, 15). They occurred in masses of 20–50. Here they usually lacked a central dense area. As individual particles it was difficult to differentiate them from some of the normal particles found in most cells including malignant ones. However, when they occur around apparently altered mitochondria

(Figs. 17–21) (26) and in masses and show little variation in size; and when they were seen in serial sections of individual cells, (Figs. 13–15) or directly below an area in which the microvilli are altered, then their identification as viral particles seems more valid.

It is possible to arrange pictures in a series showing several stages of changes, from normal mitochondria to fluid-containing vesicles in which individual virus particles occur at the edge of the distorted mitochondria (26). In several instances we have found intact mitochondria with one or two particles within them. However, when there are enough of the particles to be sure of them, the mitochondria are so altered that in turn their identification is open to question. The mitochondria in the part of the cell not in contact with these clusters looked normal but were usually absent from the area of direct contact with the clusters of particles.

The interpretation of the virus-like particles rests on continued comparison with other types of cells, particularly with normal mammary gland cells and with other kinds of tumors. The variety of changes found in some other mammary tumors of mice, both with and without the inciter, is included in the second paper.

Mammary glands from C_3H mice with and without the inciter were studied. These were studied both from virgin and lactating mice. Due to the high fat content of this tissue and the less compact nature of the cell matrix it has been difficult to obtain sections that are as satisfactory as the ones of malignant tissues. The normal mammary gland has at no time shown particles easily confused with the virus particles.

Most lactating cells have at the surface numerous microvilli which are usually regular in length and diameter (Figs. 22, 23). Scattered lipoid particles of varying sizes are found within cells (Fig. 26) and within the lumen of the glands. The process of secretion appears to consist in a breakdown of peripheral portions of the cell so that all of the particulates, including mitochondria, escape into the lumen (Fig. 25). The milk is composed of two types of particles which are free within the lumen: large dense lipoid droplets often equal in size to the nuclei of the cells from which they come, and smaller droplets varying in density and size, sometimes less than 50 millimicrons. They are uniformly homogeneous, have perfectly sharp borders, and vary greatly in size within one group. They have no apparent relationship to the microvilli of the cells. They seem to escape from the secreting cell by breaking through the cellular membrane. The cytoplasm of the secreting cells contains numerous large droplets of both the uniform dense lipoid kind and of the smaller type just described. The small granules associated with the endoplasmic reticulum are rather prominent. We have failed to see any virus-like particles in the cytoplasm in these cells.

Relatively few cells were seen in the normal virgin mammary gland sections (Figs. 24, 27, 28). Many of these were apparently fat cells. The occasional secretory cell contained large mitochondria and a few lipoid droplets.

TABLE I

Attempted Neutralization of Varying Dilutions of M.T.I. by Undiluted Pooled Mouse Sera

Dilutions of Virus	Pooled Sera from:					
	Agent ♀ C3H mice with tumor		Agent ♀ C3H mice before tumor		Non-agent ♀ C3H mice	
	Proportion with tumor	Time tumor developed	Proportion with tumor	Time tumor developed	Proportion with tumor	Time tumor developed
		months		*months*		*months*
10^{-5}	5/6	15–22	4/7	15.5–22	4/4	10–14.5
10^{-4}	7/7	14–22	1/1	15	2/3	14 and 15
10^{-3}	1/3	19	4/4	14–20	3/3	15–21
10^{-2}	6/9	18–22	3/4	17–20	3/4	14–19
10^{-1}	1/3	20	2/5	17 and 20	2/7	15 and 22
Undil.	3/6	14–22	1/4	20	0/3	

TABLE II

Attempted Neutralization of Varying Dilutions of M.T.I. by Undiluted Pooled Mouse Sera

Dilutions of Virus	Pooled sera from:					
	Agent ♂ C3H mice		Non-agent ♂ C3H mice		Agent ♀ C3H (Virgin)	
	Proportion with tumor	Time tumor developed	Proportions with tumors	Time tumor developed	Proportion with tumor	Time tumor developed
		months		*months*		*months*
10^{-5}	2/4	13 and 21	0/1		4/6	12, 14 and 21
10^{-4}	3/5	11, 18 and 20	3/3	19 and 20	2/4	17 and 19
10^{-3}	0/2		3/4	14–19	2/7	15 and 11
10^{-2}	5/5	8–21	2/3	17	0/1	
10^{-1}	3/4	11, 17 and 22	3/6	11.5, 19 and 20	4/6	18–20
Undil.	1/5	20			1/5	21

PROTOCOL FOR TABLES

Differentially centrifuged preparations obtained from saline extracts of tumors were diluted in buffered saline. The various dilutions of "virus" were then combined with the different preparations of undiluted inactivated pooled sera. The combinations were placed at room temperature for 30 minutes and then with separate needles and syringes each dilution combination was inoculated intraperitoneally into two litters of newly born (1–3 days) mice from a line of C3H mice known to be free of the agent of mammary tumor. Later the male members of these litters were discarded. Mice inoculated at each dilution were kept separately and raised. They were subjected to two or three pregnancies and then kept until the age of twenty two months or until they developed tumors. Each tumor was sectioned for histological study and the presence of mammary tumor established.

We have several times referred to the necessity that large numbers of virus particles be present in a tissue before it is possible to find and identify them in thin sections of tissues (6, 27). Since the particles here described occurred in moderate numbers it appeared possible that there was sufficient virus present to produce hemagglutination. Furthermore it was necessary to determine directly what dilution of the tumor suspensions was able to infect new mice.

Differential centrifugation designed to separate particles of about 70–100 mμ was applied to a series of spontaneous tumors. The slightly cloudy fluid

derived thereby from the tumor was then heated in different tests at temperatures from 56° to 75°C. for 30 minutes. This was in the hope that combined virus might be liberated (28). The unheated and heated preparations were then tested for their capacity to agglutinate mouse red cells. The heated preparations were found capable of agglutinating red cells, but only if the somewhat coarse particles which had been formed by the heating of the differentially centrifuged material were kept suspended. Furthermore, preparations of liver from both normal and infected (MTI) mice also were capable of causing agglutination of mouse red cells under these conditions. Finally pooled sera from mice which carried the agent (whether with or without tumor) and mice which did not have the agent were equally capable of inhibiting the agglutination of these cells. There was therefore no evidence that the hemagglutinating activity was related to the presence of the virus.

Titrations of the agent were performed as part of two attempted neutralization tests. In Tables I and II the results of these titrations are presented. It may be seen that a 10^{-5} dilution of the material derived from the 15 per cent suspension of the tumor produced infection in both experiments in most of the mice. Thus a 10^{-6} dilution of the original tumor produced tumors when 0.05 cc. was inoculated intraperitoneally in baby mice. The tumors then contained more than 10^{7} infectious particles per gram of tissue. This makes it likely that the tumors do contain sufficient virus for visualization in the electron microscope. This becomes more likely if it takes more than one virus particle to initiate infection in a mouse.

DISCUSSION

No new significant difference between the normal and the malignant cells were found except for the presence of particles which we have tentatively identified as virus particles. As we have indicated elsewhere, no single criterion is satisfactory for such identification, and it is difficult to identify a given particle as a virus particle (27).

The uniform occurrence of clusters of empty sphere-shaped particles in characteristic locations in tumors, particularly near the interface between the mitochondria and the cytoplasm, their presence inside microvilli where they appear as dense centers within the tips of the microvilli, and their uniform size and arrangement encouraged us to identify them as virus particles. Our failure to find them in virgin or lactating mammary glands of C_3H mice, whether the agent was present in the line or not, may merely mean that there were not enough of them to be caught in thin sections.

The appearance of the particles deep in the cell, particularly around the vesicles, differed from the appearance of those particles found as clusters either at the surface of the cell or within the microvilli protruding from the cell. The particles occurred as small empty spheres within the cytoplasm, whereas they appeared as dense particles in the villi and outside. The occasional oval appear-

ance is no doubt due to compression during cutting. This change in appearance has been described for several other well identified viruses (29, 30) and is not adequately understood. It is not known whether a developmental stage of the virus is represented or whether virus within the cell is subject to artifactual changes not apparent in virus outside the cell.

It is our impression that these particles grow within the cytoplasm of the cell and frequently concentrate at the mitochondrial-cytoplasmic intersurfaces, and that they are extruded from the surface of the cell either as individual particles in altered microvilli or in clusters from a disintegrating surface of a cell. Occasional ones are pushed into vesicles within the cell or less commonly into mitochondria. When extruded they increase in size, possibly taking up host cell cytoplasm.

The intracytoplasmic particle is then about 70 mμ in size whereas the extruded particle has increased in size. The dimensions of the particles studied here correspond closely to those reported by Porter and Thomson who found the particles of 130 mμ apparently occurring in the ectoplasmic portions of the cultured cells. These contained dense centers averaging about 70 mμ.

SUMMARY

1. Spontaneous mammary tumors from six C_3H mice known to be infected with the viral agent known as Mammary Tumor Inciter were studied in thin sections by electron microscopy.

2. All of these tumors showed cells which did not differ in their general architecture from normal cells. The general acinar structure was maintained and frequent interlocking processes between neighboring cells were found.

3. The fine structure of the chromosomes seen in one mitotic figure had minute individual dots and threads. The dots may represent cross sections of the threads. The nucleus at the periphery had similar fine dots more concentrated at the surface of the nucleus. A greater concentration of these toward the center of the nucleus formed the nucleolus.

4. All of the tumors had focal accumulations of particles within the cytoplasm. These were uniform in size and were found in clusters, sometimes at the apparent mitochondrial-cytoplasmic interface, frequently as small vesicles within the otherwise intact cytoplasm. Usually more dense particles were found in the tips of microvilli and extruded from the cytoplasm into the intercellular fluid. None was found within the nuclei.

5. Normal mammary glands have microvilli both in the lactating cells and the non-lactating. These do not contain such particles. The process of milk secretion apparently consists of a partial breakdown of the periphery of the cell so that a great number of particles of varying size and density accumulate and are extruded into the lumen of the gland. None of these milk particles was similar in appearance to the "virus" particles of the tumors.

6. Extracts of tumors when subjected to differential ultracentrifugation and

subsequently heated (40°C–75°C) for 30 minutes agglutinated mouse red cells. However, similar extracts of normal (uninfected) liver yielded an identical hemagglutinin.

7. In two attempted virus neutralization tests, it was shown that the infectious agent titers to more than 10^7 per gram of tissue.

We are indebted to Mr. Vytautas Skibniauskas for the photographic work and to Mrs. F. B. Bang for the serial section reconstruction.

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PLATE I

FIG. 1. Low power micrograph of typical adenocarcinoma with acinar structure. Capillary at upper right shows thin expanse of epithelium and dense red cells within. Minute particles within central area are the suspected mammary tumor agent. × 4,070.

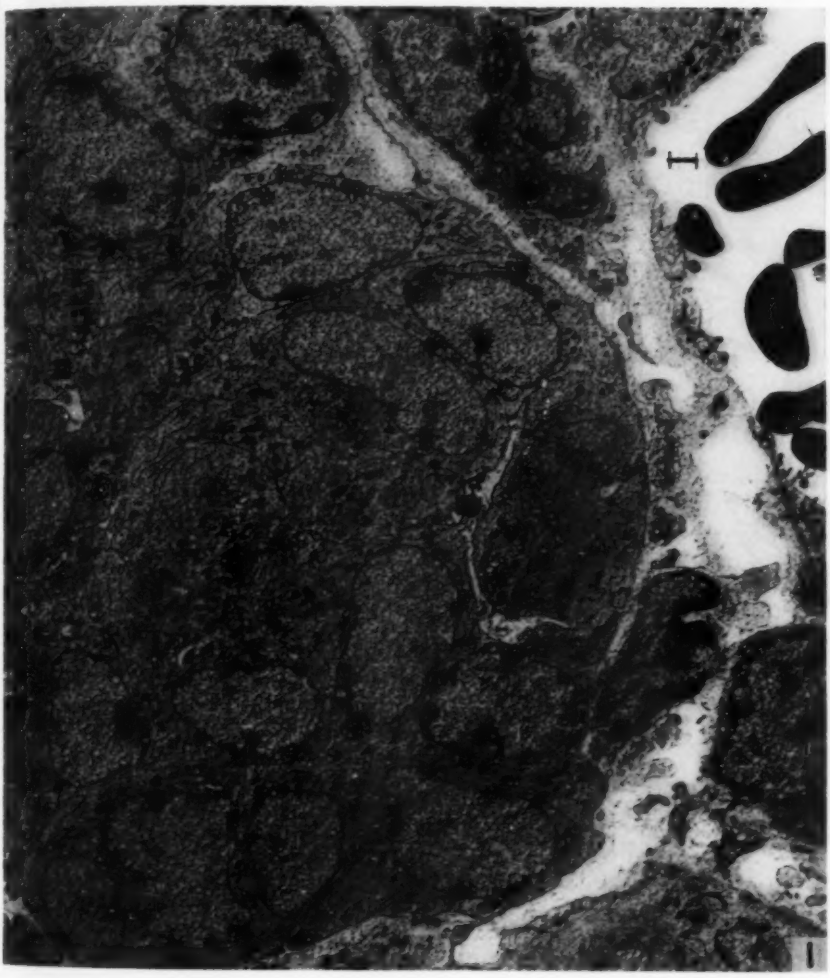
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PLATE II

FIG. 2. Interdigitating processes of three tumor cells. Nuclear membrane both in cell at right and left of illustration, is apparently double. × 22,000.

FIG. 3. Higher magnification of another portion of "nuclear membrane". Here the separateness of the cytoplasmic and the nuclear membrane is more apparent. × 38,000.

2

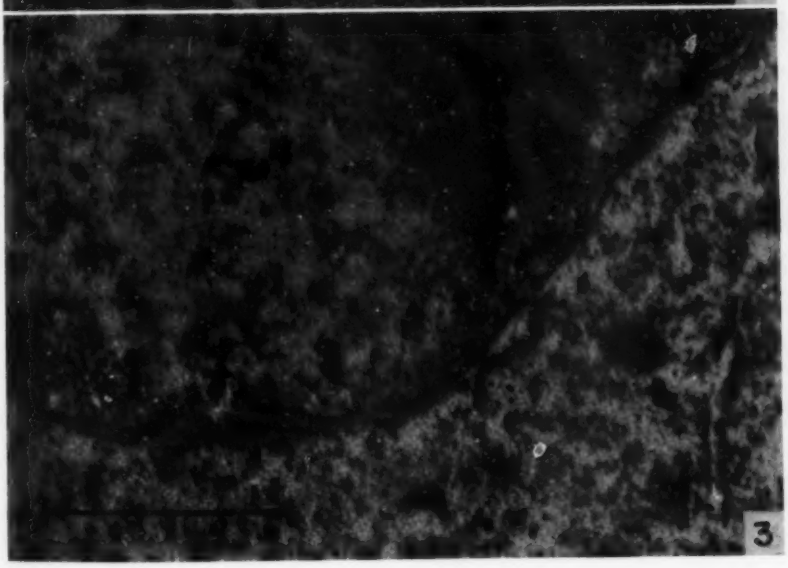
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PLATE III

FIG. 4. Low power micrograph of mitotic figure of spontaneous adenocarcinoma. C_3H mouse. × 8,280.

FIGS. 5 and 6. Serial sections of central area of same figure. The granular nature of chromosome material is apparent. However, it is not possible to determine to what extent this represents cross sections of threads and to what extent they are true granules. Darker components measure about 20 mμ in diameter. Amorphous material in background. These granules are similar in size and shape to these in the nucleolus of Figure 3. × 43,760.

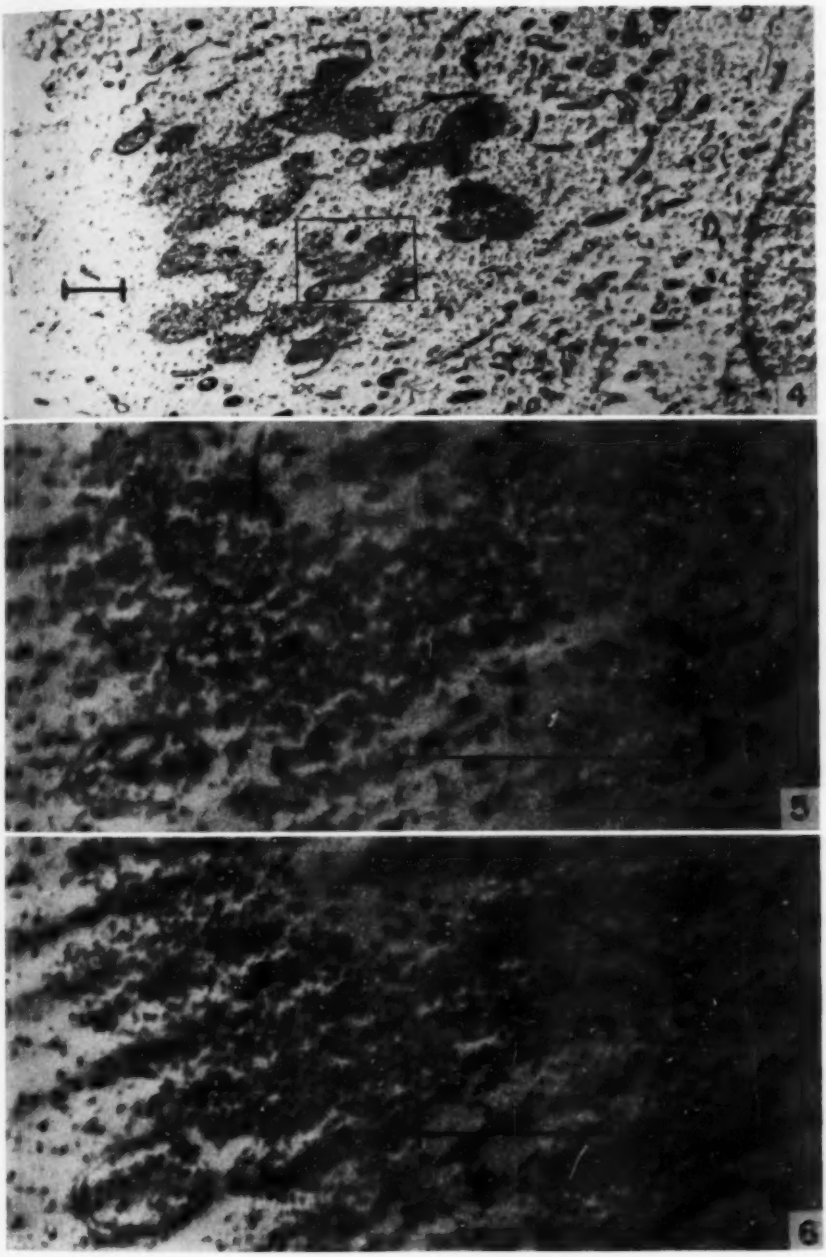
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PLATE IV

FIG. 7. Typical particles of presumed virus in the tips of altered microvilli and apparently free in the middle of vesicular collection of fluid. Extra cytoplasmic particles indicated by arrows show empty sphere-shaped appearance within, thus impression of double walled structure is produced. C_3H spontaneous tumor in 6 month old mouse. × 32,600.

FIG. 8. Another area from the same tumor. A small cluster of the particles as they appear in cytoplasm is indicated at right border. × 21,880.

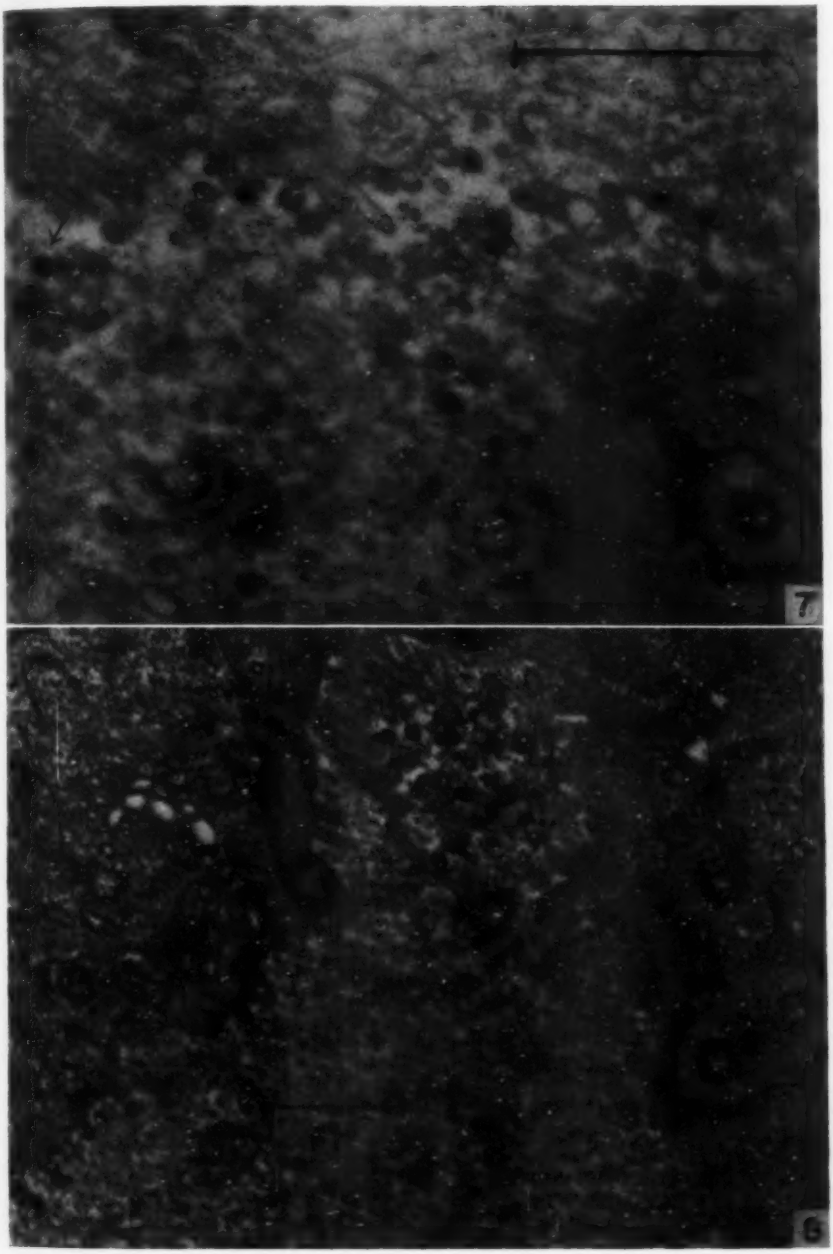
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PLATE V

FIGS. 9 and 10. Somewhat thicker section of two vesicles with characteristic particles projecting in several different kinds of microvilli. Figure 10 shows normal appearing mitochondria of cell next to vesicle. Mammary tumor which occurred 15 months after the injection of mammary tumor inciter in agent free C_3H new born female. × 21,880.

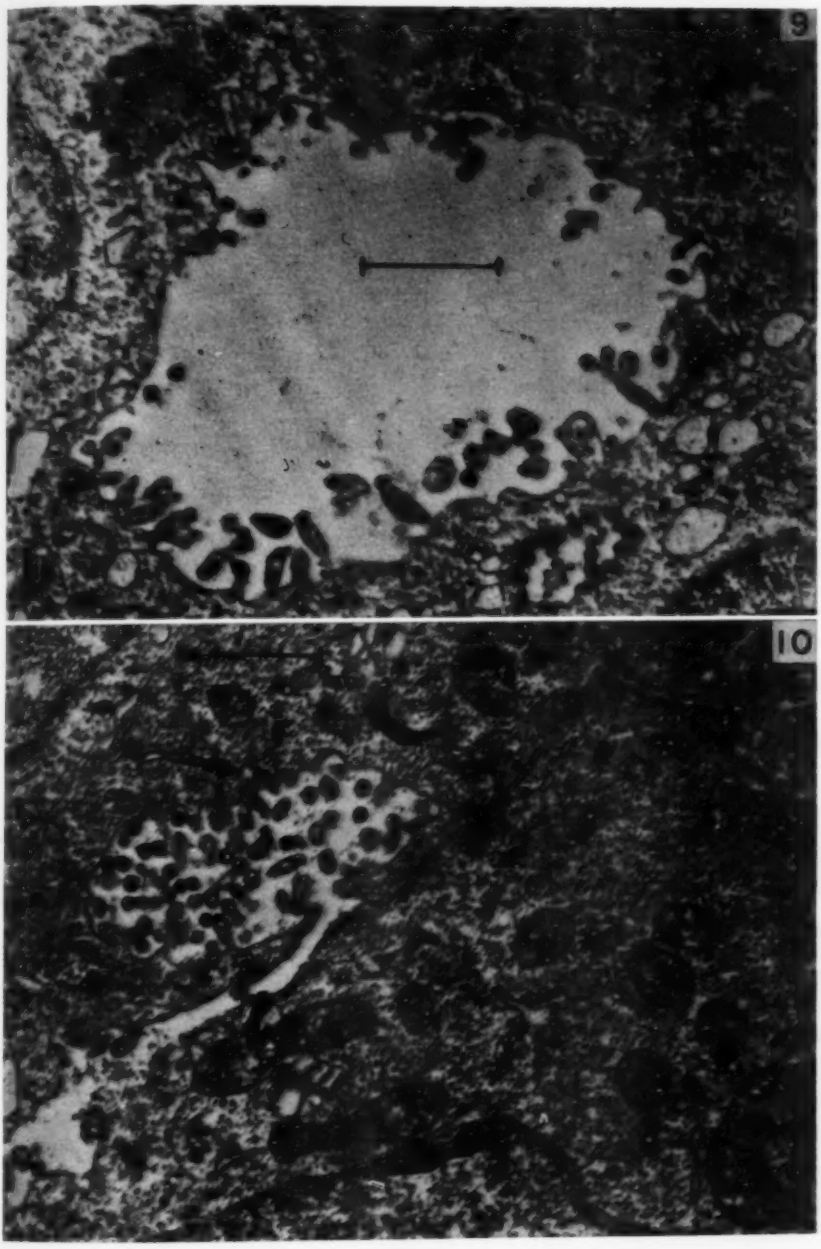
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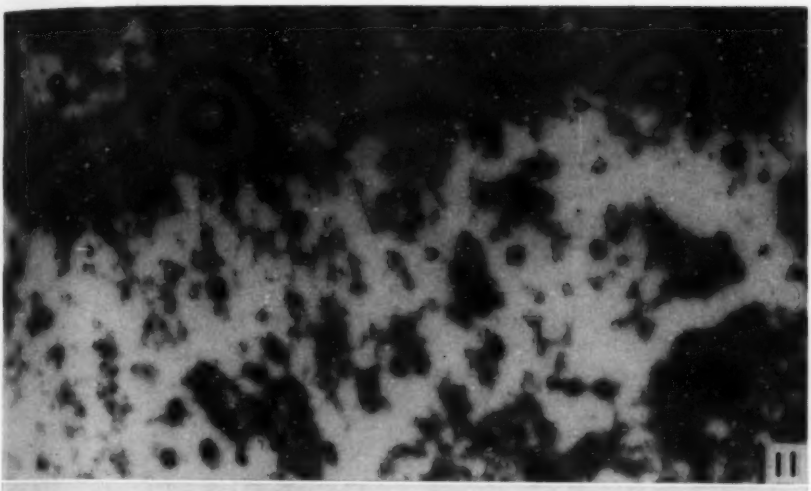
PLATE VI

FIG. 11. One section of vesicle which was cut serially and then reconstructed as in Figure 12. A scattering of small empty sphere shaped particles are seen in the cytoplasm in the upper portion of Figure 11. × 21,880.



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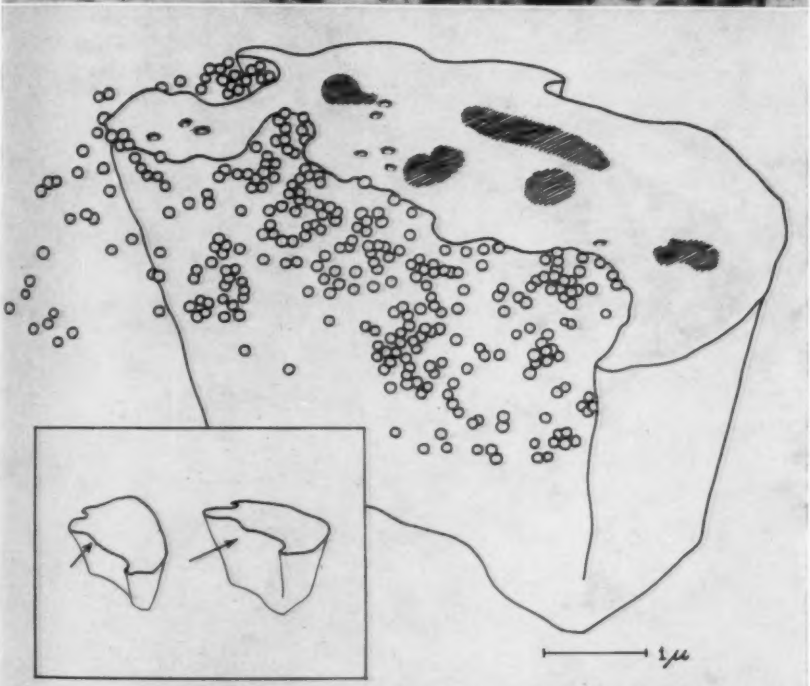

Diagrammatic Reconstruction of 14 serial sections of mouse mammary tumor cell

12

PLATE VII

FIGS. 13, 14 and 15. Serial sections of a vesicle in a spontaneous mammary tumor occurring in a female at 9 months of age. Milk agent present. A number of these particles are thus demonstrated to be free in the fluid and no longer attached to microvilli. However, original coating apparently derived from host cell is still present. In lower left portion of each figure smaller hollow particles are seen in characteristic clumps. × 21,880.

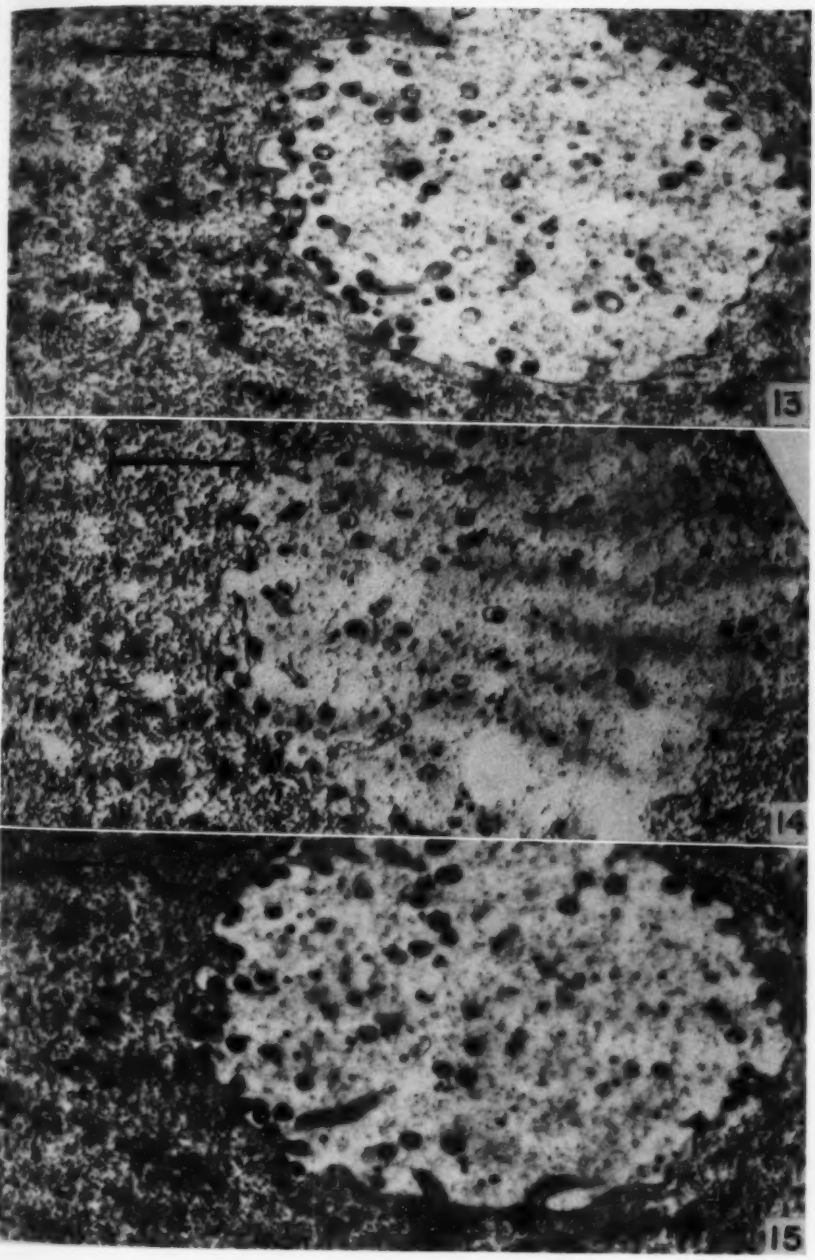
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PLATE VIII

FIG. 16. Low power view of another area of same tumor as illustrated in Figures 9 and 10. × 4,140.

FIGS. 17–21. In upper portion of figure is a homogeneous oval body with several particles with it. This area may be followed in the subsequent sections and it can be seen that small oval particles are surrounding a clear vesicular area which corresponds to the original homogeneous one. There is a large interruption between Figures 18 and 19. Figures 19, 20 and 21 are serial. Large dense sharply defined black crystals are artifacts. × 20,500.

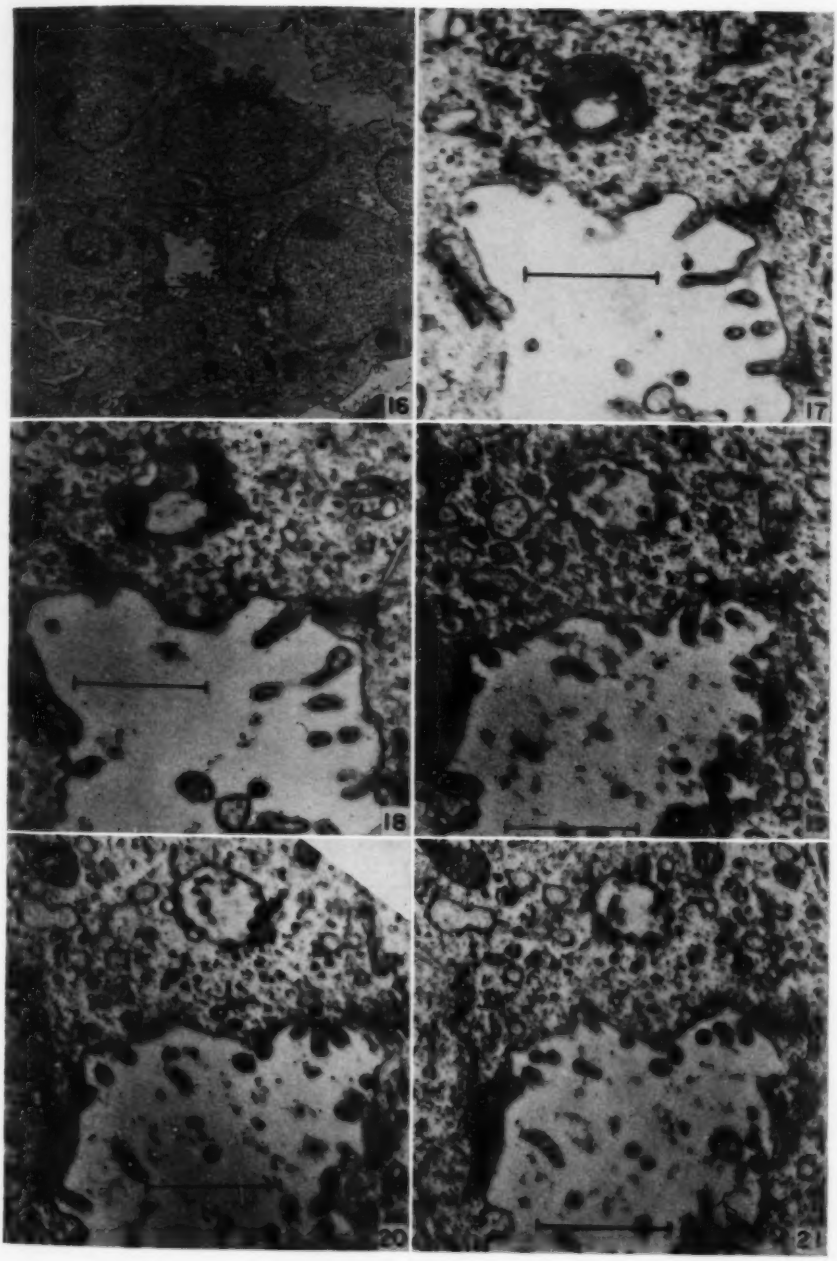
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PLATE IX

FIGS. 22 and 23. Thick sections of normal lactating mammary gland. Numerous blunt microvilli projecting into lumen of gland. Large dense black inclusions presumed to be fatty inclusions as they break free into cell. Irregular shape of these probably an artifact of fixation or embedding. Cutting artifacts also pronounced in these. × 5,300.

FIG. 24. Normal virgin mammary gland. Edge of two cells in thick section showing numerous microvilli. × 14,360.

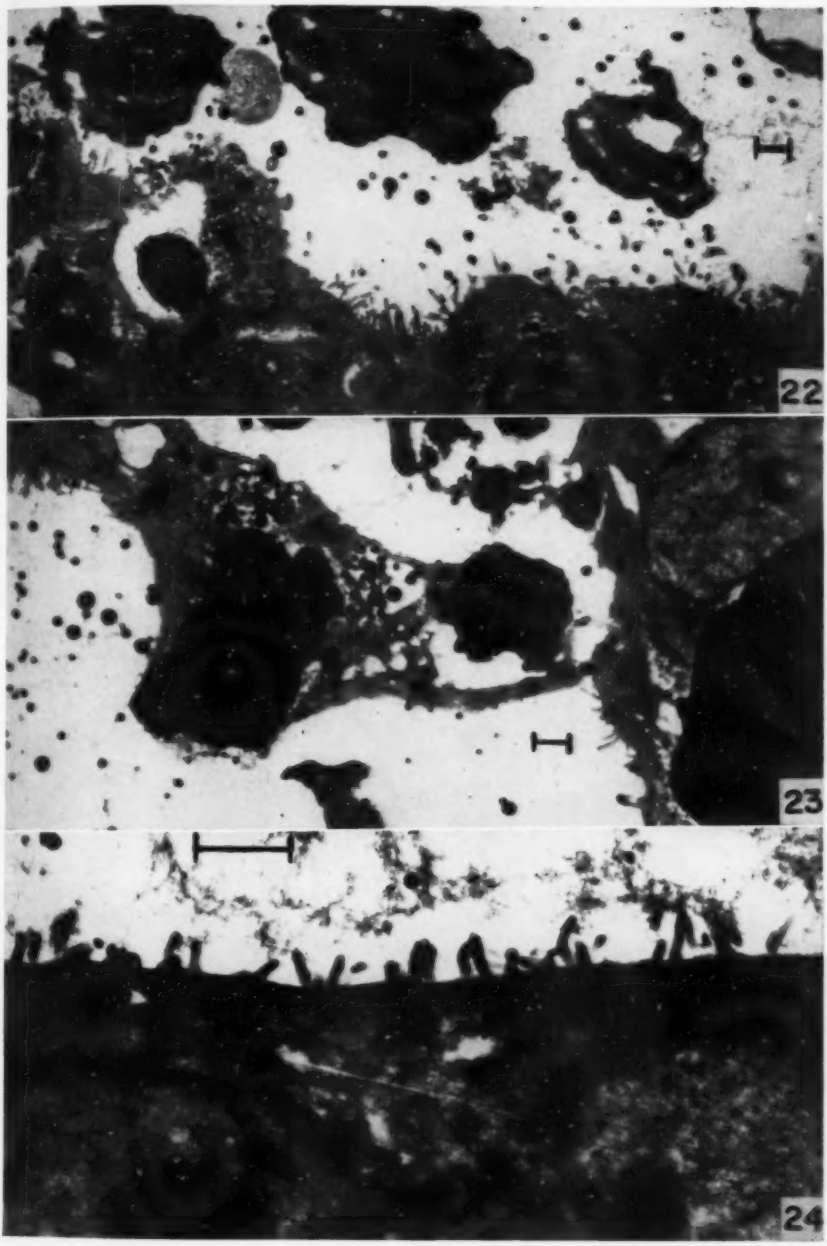
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PLATE X

FIG. 25. Normal lactating gland. Thin section at edge of lactating cell. Easily identifiable mitochondria escaping along with cellular debris and few definite fat droplets. Clear areas throughout cell may represent inadequately fixed fat which has been dissolved out of the section. Electron microscopy thus confirms ideas of cell breakdown during lactation. (31) × 14,590.

FIG. 26. Capillary within normal lactating gland. Several red cells cut in section. Irregular projection into lumen from these endothelial cells is not found in capillary endothelium of virgin mammary glands. Compare with Figure 28. × 6,100.

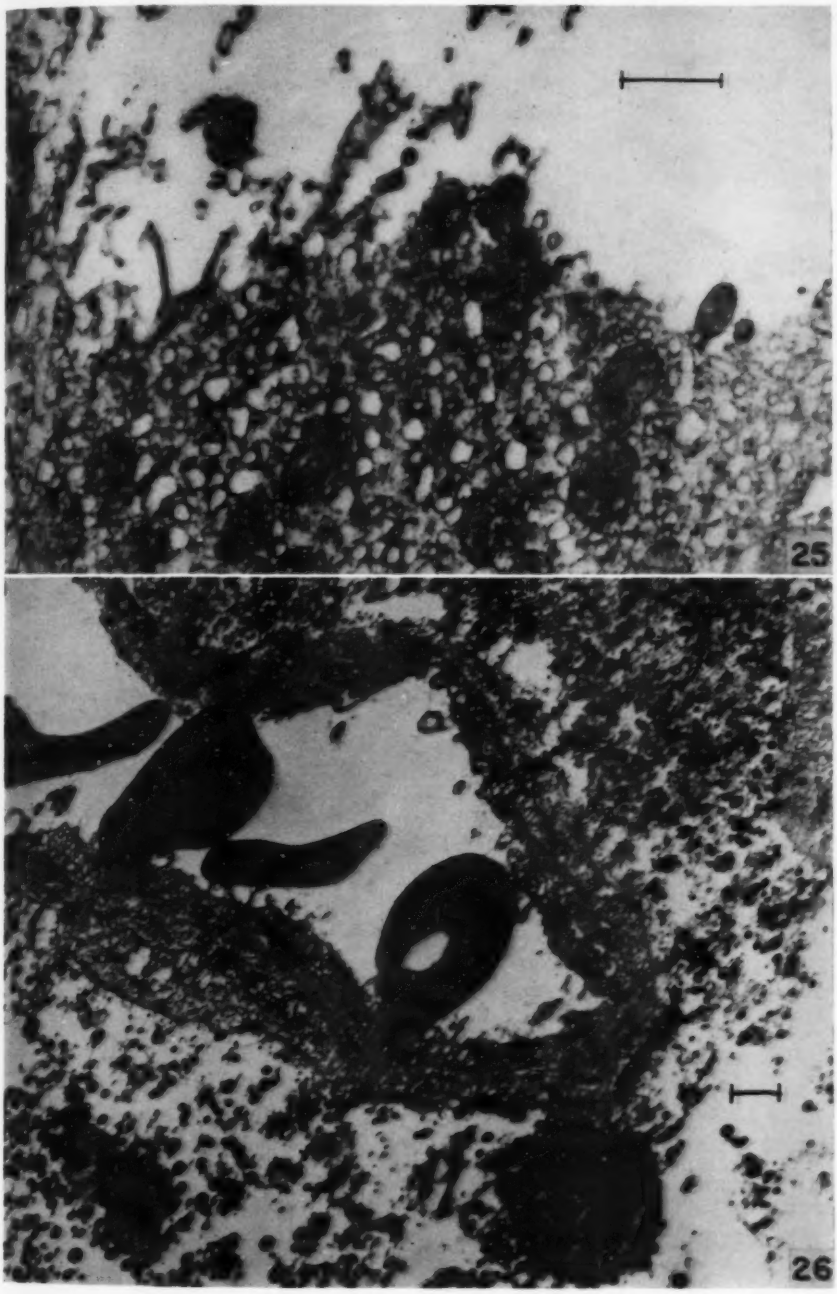
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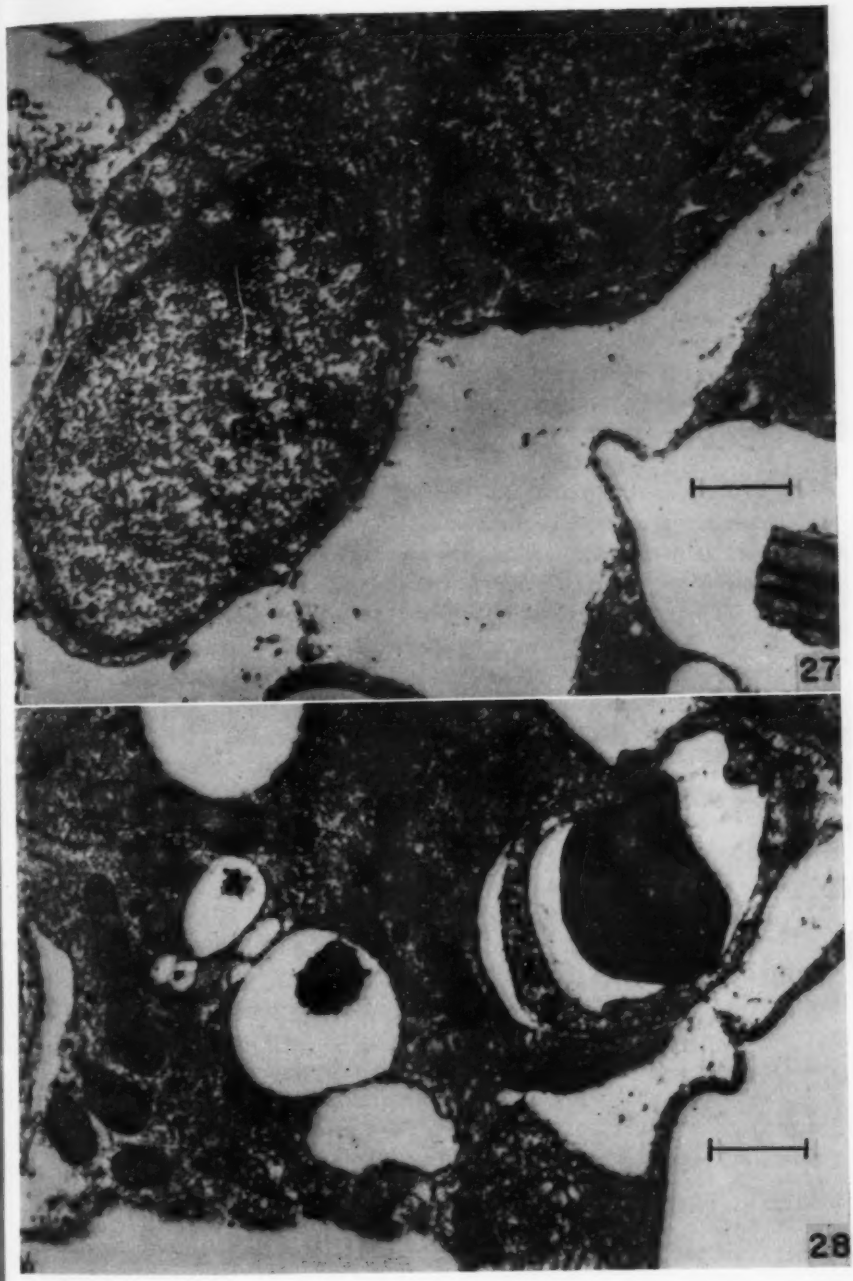
PLATE XI

FIG. 27. Two glandular cells from mammary gland of normal virgin mouse. Compare appearance of these cells with those of spontaneous tumors. Wide clear areas here are due to fat which has retracted during fixation. This has left the thin shell of the cytoplasm of the fat cell seen just to left of micron mark. × 14,700.

FIG. 28. Normal virgin mammary gland. Capillary with red cell inside. Normal dense mitochondria and retracted fat droplet within cell which presumably is glandular. × 14,700.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ELECTRON MICROSCOPIC EVIDENCE CONCERNING THE MAMMARY TUMOR INCITER (VIRUS)

II. An Electron Microscopic Study of Spontaneous and Induced Mammary Tumors of Mice

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The previous paper (1) described certain particles within the cytoplasm of mammary tumor cells from strain C_3H mice known to carry the mammary tumor inciter (MTI). These particles were not found in mammary gland cells of normal mice whether they were or were not lactating.

As a means of evaluating this finding forty-one mouse mammary gland tumors were studied. The tumors arose in mice of various genetic constitutions, some of which were known to carry the inciter while others were presumably free of the inciter. Tumors were studied in the electron microscope without prior knowledge of the cause or primary stimulus of a given tumor. In Table I we have listed the results and compared the findings.

Of the forty-one tumors, thirteen showed results in which the electron microscope findings and the biological data disagreed. In eight of these the inciter was not found by electron microscopy when it was known to be present. In five tumors particles were found which resembled those previously identified as the inciter, though biological data indicated that it was not present in the hosts.

Several of the tumors deserve more thorough description. However, electron microscope study of each tumor involves such a small sample of the total, and the morphology may vary somewhat within the tumor, so that it is difficult to evaluate the individual findings.

Section 238 (Fig. 1). This tumor arose in an 8.5 month old F_1 female derived from a strain C female and a strain DBA/2 male. The mammary tumor inciter had been injected intraperitoneally 13 days after birth. Although this section was cut with the Spencer microtome and is thus considerably thicker than many subsequent ones it shows the location of the particles with their dense and frequently eccentric center. Long thin filaments presumed to be microvilli are also present. As has been emphasized in studies of other viruses, when they

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[1] National Institutes of Health, U. S. Public Health Service, Department of Health, Education and Welfare.

TABLE 1

A Comparison of Electron Microscope Findings with Origin of Mice that Developed Tumors

Mice						Electron Microscope Findings	
Section No.	Genetic constitution	Sex	Age at tumor	Treatment and remarks	Biological indication for presence of MTI*	Diagnosis	Remarks
			months				
214	R III	F	5.5	None; breeder from colony	+	+	Microvilli with particles at tip. Central density
215	DBA/2	F	6.5	Methylcholanthrene orally; stilbestrol subcut.; MTI free	−	−	Dense scattered cells; no microvilli or particles
216	DBA/2	F	8	Methylcholanthrene orally; stilbestrol subcut.; MTI free	−	−	Coiled complex nucleoli; no particles
217	DBA/2	F	6.5	Methylcholanthrene orally; stilbestrol subcut.; MTI free	−	−	Compact cells; no vesicles or particles
218	C_3H	F	10	None; virgin from colony	+	+	Vesicles and microvilli with dense particles at tips
219	C_3H	F	10	None; virgin from colony	+	+	Poor pictures but vesicles and microvilli and dense centers are apparent
230	R III	F	6.5	None; breeder from colony	+	+	Compact tumor; diffuse nucleoli, scattered clumps of particles in clear areas with dense centers
231	R III	F	6.5	None; breeder from colony	+	+	Poor pictures; 2 areas with vesicles and particles
233	$(C \times DBA/2)F_1$	F	7	MTI intraperitoneally when 11 days old; bred	+	+	Poor pictures; scattered doubtful particles
234	$(C \times DBA/2)F_1$	F	13	Carried wild mouse MTI; bred	+	−	Poor pictures; nothing suggestive seen
235	$(C \times C_3H)F_1$	M	9	Castrate; stilbestrol subcut.; mother free of MTI	−	−	Poor picture; nothing suggestive seen
236	$(C \times DBA/2)F_1$	F	13	Carried wild mouse MTI; bred	+	−	Poor picture; nothing suggestive seen
237	$(C \times DBA/2)F_1$	F	7.5	MTI intraperitoneally when 13 days old; bred	+	+	Small vesicles with microvilli and fairly typical particles
238	$(C \times DBA/2)F_1$	F	8.5	MTI intraperitoneally when 13 days old; bred	+	+	Good long microvilli with scattered typical particles; also clusters at surface of cell
239	$(C \times C_3H)F_1$	M	13	Castrate; stilbestrol subcut.; mother free of MTI	−	−	Poor pictures; nothing seen

238	(C × DBA/2)F₁	F	8.5	MTI intraperitoneally when 13 days old; bred	+	+	Good long microvilli with scattered typical particles; also clusters at surface of cell
239	(C × C₃H)F₁	M	13	Castrate; stilbestrol subcut.; mother free of MTI	−	−	Poor pictures; nothing seen
240	(C × C₃H)F	M	10.5	Castrate; stilbestrol subcut.; mother free of MTI	−	−	Scattered doubtful particles; nothing clearly positive
241	(C × C₃H)F₁	M	12.5	Castrate; stilbestrol subcut.; mother free of MTI	−	−	Good pictures; distended mitochondria; coiled nucleoli; good endoplasmic reticulum
242	C	F	14	Carried wild mouse MTI; bred	+	+	One small vesicle with typical particles
243	(C57 BL × I)F₁	F	10.5	MTI intraperitoneally when 14 days old; bred	+	−	Dense pictures with poorly defined particles
528	C	F	12	Carried wild mouse MTI; bred	+	+	Scattered typical clumps of particles in cytoplasm with destruction of cytoplasm; particles also inside of vessels
530	(C₃H × C)F₁	M	14	Castrate; stilbestrol subcut.; mother free of MTI	−	+	Extensive necrosis of tissue. Scattered particles at surface. Many particles with microvilli with dense centers
531	(C₃H × C)F₁	F	19.5	Stilbestrol subcut.; mother free of MTI	−	+	Vesicles with typical particles with dense center
532	R III	F	9.5	None; breeder from colony	+	−	Compact cells; some with coiled nucleoli. No particles seen
533	(C₃H × RIII)F₁	F	17.5	Stilbestrol subcut.; mother free of MTI	−	+	Small vesicles; typical particles with dense centers
534	R III	F	5.5	None; breeder from colony	+	−	Good pictures; no vesicles, microvilli or particles
535	C₃H	M	20.5	Castrate; stilbestrol subcut.; mother free of MTI	−	−	Peculiar cells with inclusions; no particles
639	(C × C₃H)F₁	F	15.5	Bred—1 litter; stilbestrol subcut.	−	−	Large elongated cells heavy endoplasm retic. No particles
640	C₃H	F	9	None; breeder from colony	+	+	Typical particles within large vesicular fluid. Serial sections with particles
641	(C × C₃H)F₁	M	11.5	Castrate; stilbestrol subcut.; mother free of MTI	−	+	Scattered dense rings in cells and few suggestive particles at surface. None typical
642	C₃H	F	7.5	Colony female; stilbestrol subcut.	+	−	Excellent sections; one series of 3 shows clump of altered mitochondria—tiny particles present in one—but none of right size for MTI

TABLE 1—*Continued*

Mice						Electron Microscope Findings	
Section No.	Genetic constitution	Sex	Age at tumor	Treatment and remarks	Biological indication for presence of MTI*	Diagnosis	Remarks
			months				
643	C_3H	F	7.5	Breeder from colony	+	+	Good sections; fair focus; scattered spherical particles away from cell; none beyond question
646	$(C \times C_3H)F_1$	M	14	Castrate; stilbestrol subcut.; mother free of MTI	−	−	One good picture; this neg.
647	C	F	12.5	Breeder from colony carrying C_3H MTI	+	−	Fair sections. No virus seen
648	C_3H	F	8.5	Breeder from colony	+	−	Good sections. No virus seen. Compact interlocking cells. Capillaries show few distended mitochondria and overlapping endothelium
649	$(C \times C_3H)F_1$	M	16	Castrate; stilbestrol subcut.; mother free of MTI	−	−	Fair sections. No virus
714	$(C \times C_3H)F_1$	F	20.5	Bred—1 litter; stilbestrol subcut.; mother free of MTI	−	−	Many good sections. Cells contain many regular small vesicles and number with irregular ballooning microvilli but no particles characteristic of MTI
715	$(C \times C_3H)F_1$	F	19	Bred—1 litter; stilbestrol subcut.; mother free of MTI	−	−	Many good sections. No virus; tiny particles; mitosis present
716	$(C \times C_3H)F_1$	F	20.5	Bred—1 litter; stilbestrol subcut.; mother free of MTI	−	+	Several good sections; one mitochondrian like 718 with suspected virus particles inside
717	$(C \times C_3H)F_1$	F	18.5	Bred—1 litter; stilbestrol subcut.; mother free of MTI	−	−	Good cells. Homogeneous inclusions. No virus particles
718	$(C \times C_3H)F_1$	F	7	Stilbestrol subcut.; mother carried MTI	+	+	Particles throughout
719	$(C \times C_3H)F_1$	F	13	Stilbestrol subcut.; mother carried MTI	+	+	Beautiful particles

* + denotes mouse carried MTI; − denotes mouse presumably free of MTI.

are associated with microvilli, it is difficult if not impossible to determine how much of a small particle is virus and how much is host cell.

Section 528 (Fig. 2). This tumor came from a strain C mouse that carried the inciter obtained from wild mice (2). Sections were prepared from two different blocks and both showed the same large number of particles occurring within the cytoplasm. However, these differed in some interesting respects form all of the other tumors we have examined. The number of particles that are apparent deep in the cytoplasm is much greater, and, there is much greater destruction of the cell's architecture. Two other mice (Sections 234 and 236) developed tumors following acquisition of the wild mouse inciter, but in both of these we failed to detect the inciter in the electron microscope.

Section 718. This tumor occurred in an F_1 hybrid obtained from a C female and a C_3H male. The mouse was seven months old and belonged to an experiment which demonstrated that the inciter can be transmitted from male to female (3). In the series of sections obtained from this tumor the presumed virus particles were arranged in formations which were much more sharply defined than those seen and described in the first paper. Since this particular tumor is directly related to the presence of the inciter, the evidence that this is the inciter is the same as in the preceding paper. Since a great many more sections were cut from this tumor we have illustrated these formations in greater detail (Figs. 3, 4, 5, 6, 7).

The arrangements in clusters around sharply defined vesicles, the occurrence of individual particles within certain sharply defined inclusions which we believe are mitochondria is especially striking. In these preparations serial sections again established the lack of continuity of the individual particles. The empty spherical shaped particles are more commonly found in the cytoplasm—and here lack a central dense portion. We do not know whether this becomes filled in as development proceeds or whether these are degenerate or incomplete forms.

In the sections of this tumor as in several tumors reported in the preceding paper the presumed virus particles are oval. This is an artifact of cutting (4), which Williams and Kallman (5) believe is due to compression of the tissue opposing the knife blade without compensatory expansion in the lateral direction. It is interesting to note that there is no evidence of such compression in the two tumors pictured in Figures 1 and 2. Both of these were cut as thicker sections in a harder matrix and with a steel knife.

TUMORS IN WHICH MORPHOLOGICAL AND BIOLOGICAL DATA DISAGREE

In eight tumors (Table I) we failed to find morphological evidence of the inciter and at the same time biological evidence made it clear that the tumor was associated with the inciter. Our failure to find the inciter in these tumors could be due either to the small number of particles or to inadequate sampling

* + denotes mouse carried MTI; − denotes mouse presumably free of MTI.

of the tumor. However, Section 642 was of particular interest because one area of degenerate mitochondria was cut in serial sections. No satisfactory particles were found in or near these (Figs. 8, 9, 10).

Serious disagreement in results occurs in four tumors (Sections 641, 533, 531, and 530) three of which are illustrated in Figures 11, 12, and 13, respectively. As may be seen in Figure 11, sharply defined particles appeared within the cytoplasm. As with other tumors they are hollow and about 60–70 mμ when inside the cell, whereas solid particles of about 100 mμ in the long diameter appear at the surface. Since these sections appeared compressed these long diameters are probably the true ones. In Figures 12 and 13 are shown rather thick sections of some of the first tumors studied. These show particles both in microvilli and within vesicles within the cell.

Tumors 530, 531 and 533 occurred in F_4 hybrid mice born to inciter free strain C_3H mothers.[2] All occurred in animals that were 14 to 19.5 months of age that had received stilbesterol pellets subcutaneously.

A variety of forms of tumor cells which correspond to the different types of tumors described by Dunn (6) were also seen. Some of their morphological variants are illustrated in Figures 14, 15 and 16.

DISCUSSION

This study was undertaken with the idea that by determining the presence or absence of certain particles in a number of mammary tumors, it would be possible to evaluate the findings of certain virus-like particles in tumors of mice known to be associated with the inciter of mammary tumors. Our first results were highly encouraging (7). Since then we have continued to find suspicious particles in the great majority of spontaneous tumors occurring at an early age in mice known to carry the inciter. These particles have several characteristics. When they occur within the cytoplasm they are small apparently dense bodies about 70 mμ in diameter. Like many pictures of the well identified vaccinia virus they have an empty central portion. When they occur free in the fluid or with a microvillus which is presumably involved in their liberation, they appear as dense centered bodies with the center corresponding in size to the unit within the cytoplasm. In cross section or indeed as separate particles it then is no longer possible to tell how much may be virus and how much host cytoplasm. The total particle is about 130–140 mμ whereas the smaller portion is 70 mμ.

Particles corresponding in all of these respects were found in four tumors (Sections 530, 531, 533, 641) from mice which were presumed to lack the inciter. In another (716) one small area showed suggestive particles within a mitochondria. These observations raised the question whether indeed our

[2] These were the only F_1 hybrid mice of the entire study that were born to MTI free strain C_3H mothers.

identification of the particles as the mammary tumor inciter was justifiable. There are at least six criteria (8) which may be used in identifying an agent as a virus particle. We believe that the first criterion, the finding of certain accumulations of distinctive particles has been fulfilled. Since Porter's and Thomson's (9) original report of tissue culture cells, several workers (10, 11, 12) have described the same particle (8, 10, 11, 12). All agree on essential points except for Kinosita et al's (10) finding of particles within the nucleus. We suspect that the nucleus pictured by them is one of the large vesicles filled with virus particles, which we have seen in many of our inciter induced tumors.

We have not found similar particles in normal mammary tissue—virgin or lactating, or in other normal or tumor cells of other kinds. The finding of similar particles in five tumors coming from mice presumably free of the inciter raises the question of the validity of the biological indications for presence of the inciter since none of these tumors was tested for the presence of the inciter.

The available evidence is only against an agent transmitted *through the milk* as one "cause" for this tumor. Furthermore we cannot rule out a latent viral agent which has no direct relation to the tumors but which localized and grew in the tumor cells.

The evidence for these particles as the viral agent rests on the consistent finding of large numbers of these characteristic particles in tumors known to be "caused" by the inciter and their absence from normal tissues. Further evidence for or against this identification must be sought before a final conclusion can be drawn.

SUMMARY

Forty-one spontaneous and induced mammary tumors of mice were studied by examining thin sections in the electron microscope for the presence or absence of certain particles which have been described in the accompanying paper. The particles were found in 14 of 22 tumors from mice which were known to harbor the mammary tumor inciter.

They were found in 5 of 19 tumors from mice which were presumably free of the inciter. Of these 5 tumors, 3 contained typical particles, 1 contained a few suggestive particles and 1 suspected particles.

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PLATE I

Fig. 1. Section 238. Relatively thick section of cluster of particles within and associated with microvilli. This section may well be a slice from the edge of a vesicle which was filled with particles. Note that there is little evidence of compression produced by the cutting. × 22,320.

Fig. 2. Section 528. Scattered particles in destroyed cytoplasm. Large amorphous rings may represent degenerate mitochondria. This tumor occurred in a strain C mouse 12 months of age who carried the wild mouse inciter. × 19,760.

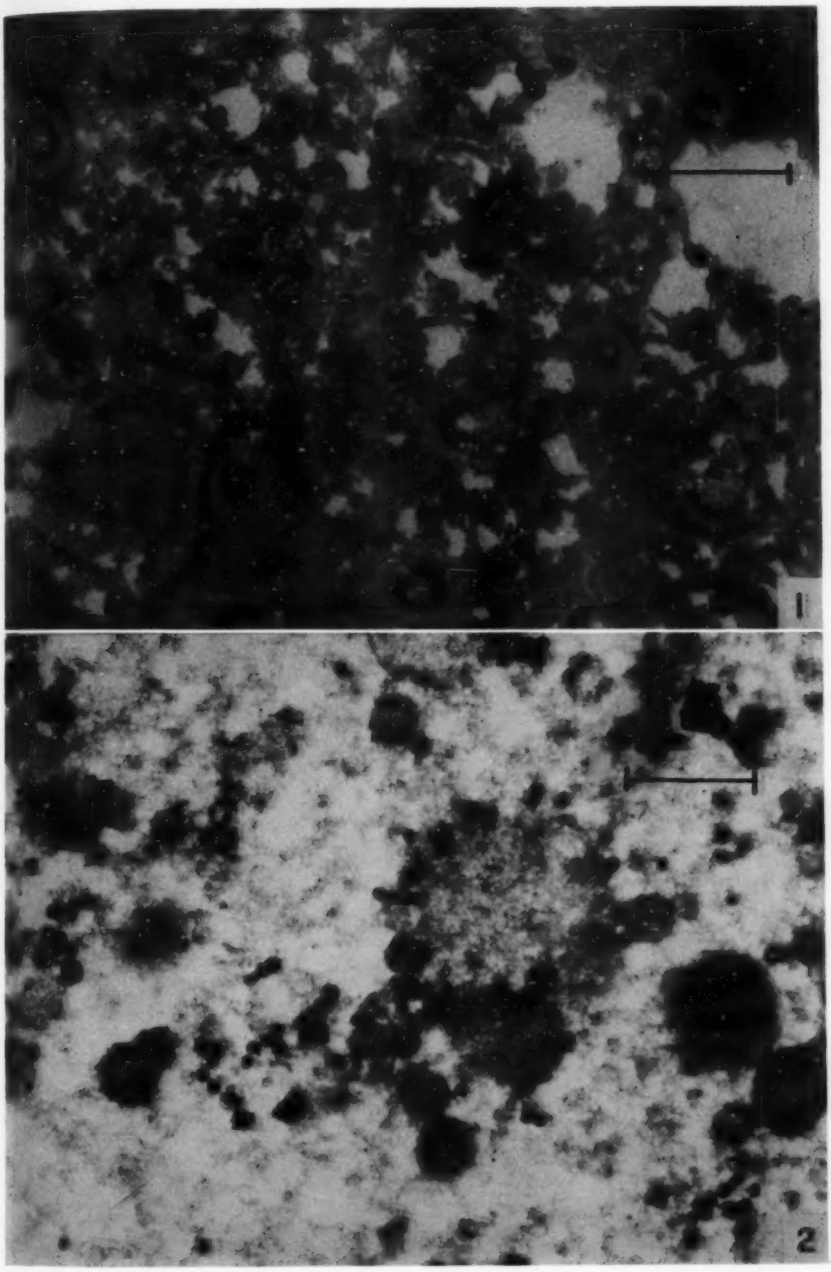
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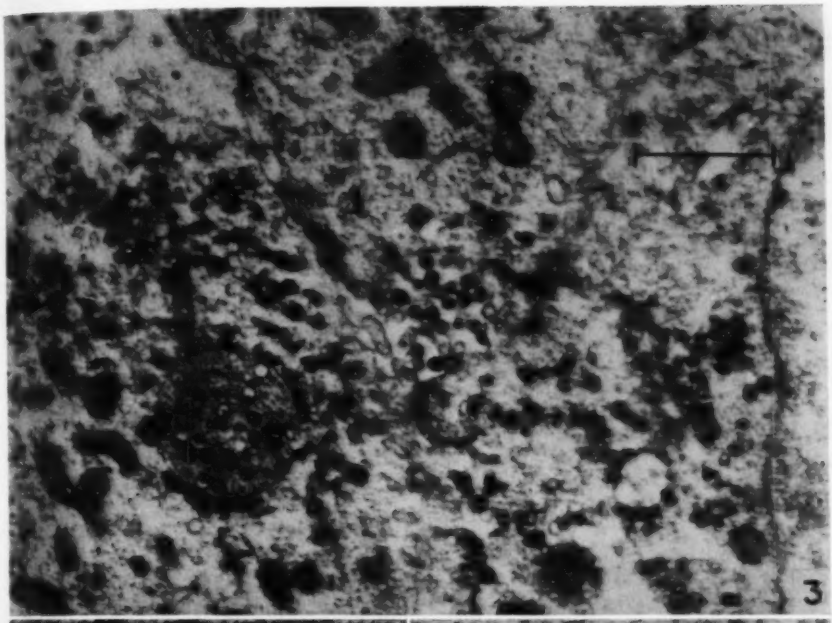
PLATE II

FIG. 3. Section 718. At left small spherical inclusion with numerous particles within. At right, near nuclear border a scattering of similar particles free in the cytoplasm. Most mitochondria in area appear normal. The tumor was from an experiment which showed male transmission of the inciter. Temperature of polymerization 60°C. × 21,780.

FIG. 4. Section 718. Large oval dense inclusion presumed to be mitochondrion. Within are two typical particles. × 21,880.

FIG. 5. Section 718. Small foci of particles, next to nucleus several particles are within vesicular collections of fluid. The smaller oval particles in the cytoplasm may be compared to the dense centers in the larger particles. × 21,880.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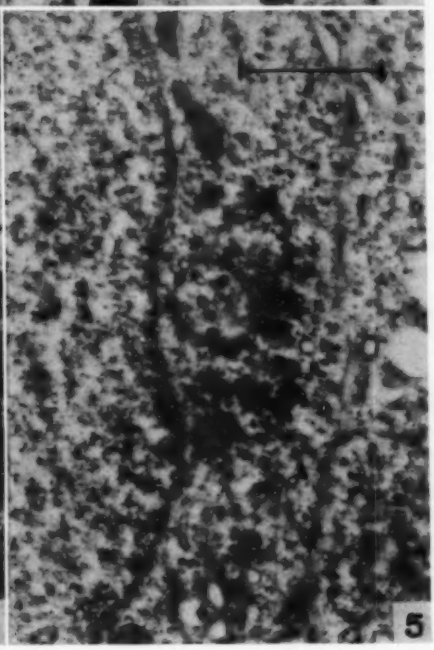
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PLATE III

FIG. 6. Section 718. Several vesicles in different stages of disintegration. Oval empty particles seen clearly outside and concentrated around edge. Few dense ones within. × 41,000.

FIG. 7. Section 718. Similar area from another portion of same tumor. Vesicle in lower portion appears very similar to the clearly defined mitochondria above, but enlarged. Other vesicles presumably present further states of disintegration. × 45,000.

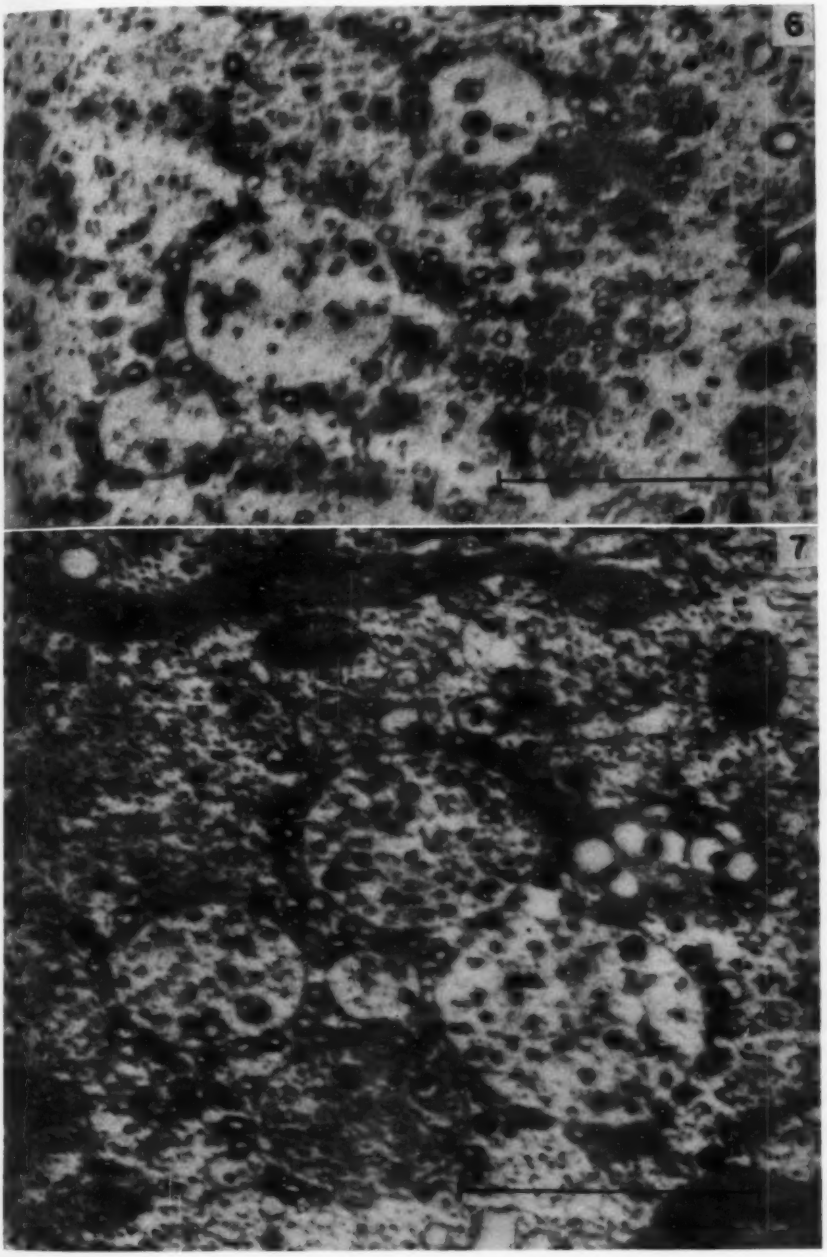
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PLATE IV

FIGS. 8, 9 and 10. Section 642. Serial sections through area of mitochondrial disorganization next to nucleus. Mitochondrion in extreme upper right has shrunk away from cytoplasm so that potential space between mitochondrion and cytoplasm is apparent. Mitochondrion just below shows particularly well the type of focal lesion which may occur in an otherwise healthy area. Scattered particles of all kinds which cannot be identified as the agent. × 21,880.

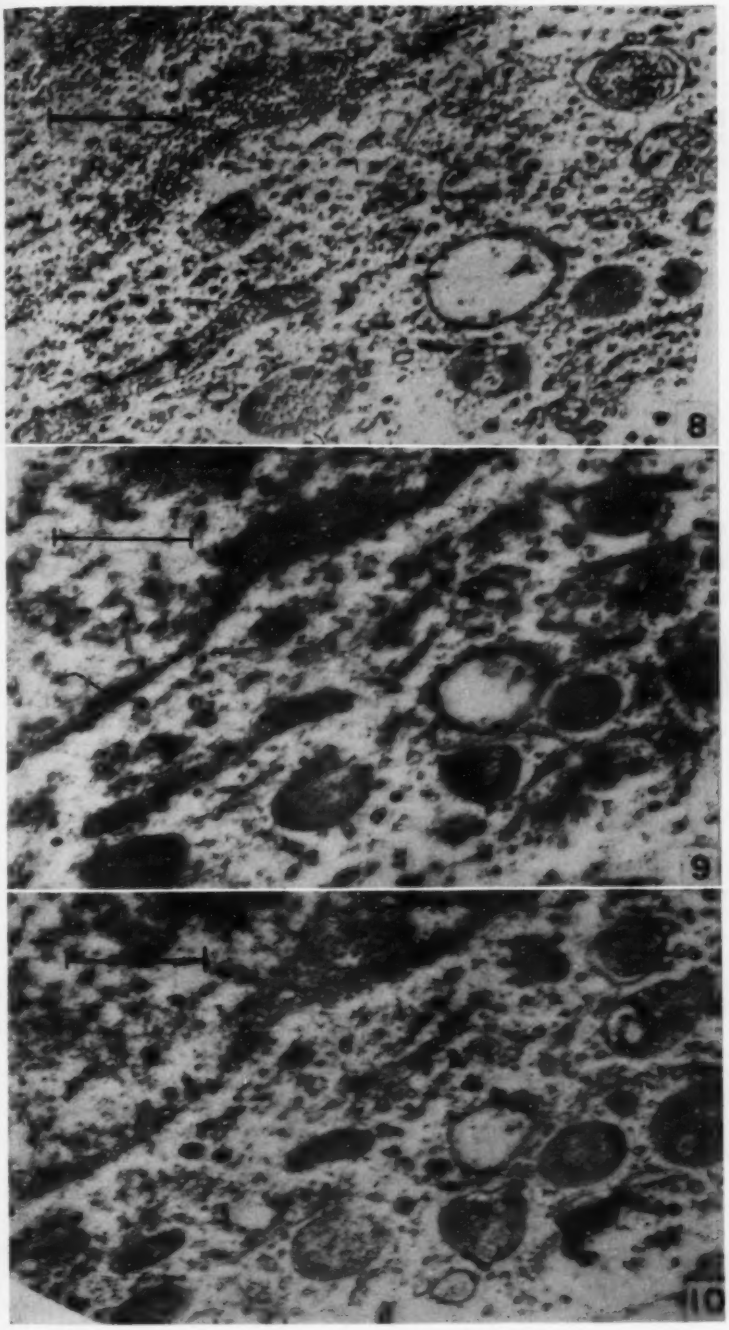
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PLATE V

FIG. 11. Section 641. Clumps of cytoplasmic particles similar in appearance to those seen in known positive tumors. Several of these at the surface show characteristic dense center with light periphery. Inciter presumably not present. × 21,880.

FIG. 12. Section 531. Small vesicular focus from thick section. Collection and arrangement of particles similar to known positive mice. × 19,760.

FIG. 13. Section 530. Similar larger focus. Microvilli may be normal but dense particles within are highly suspicious. × 19,760.

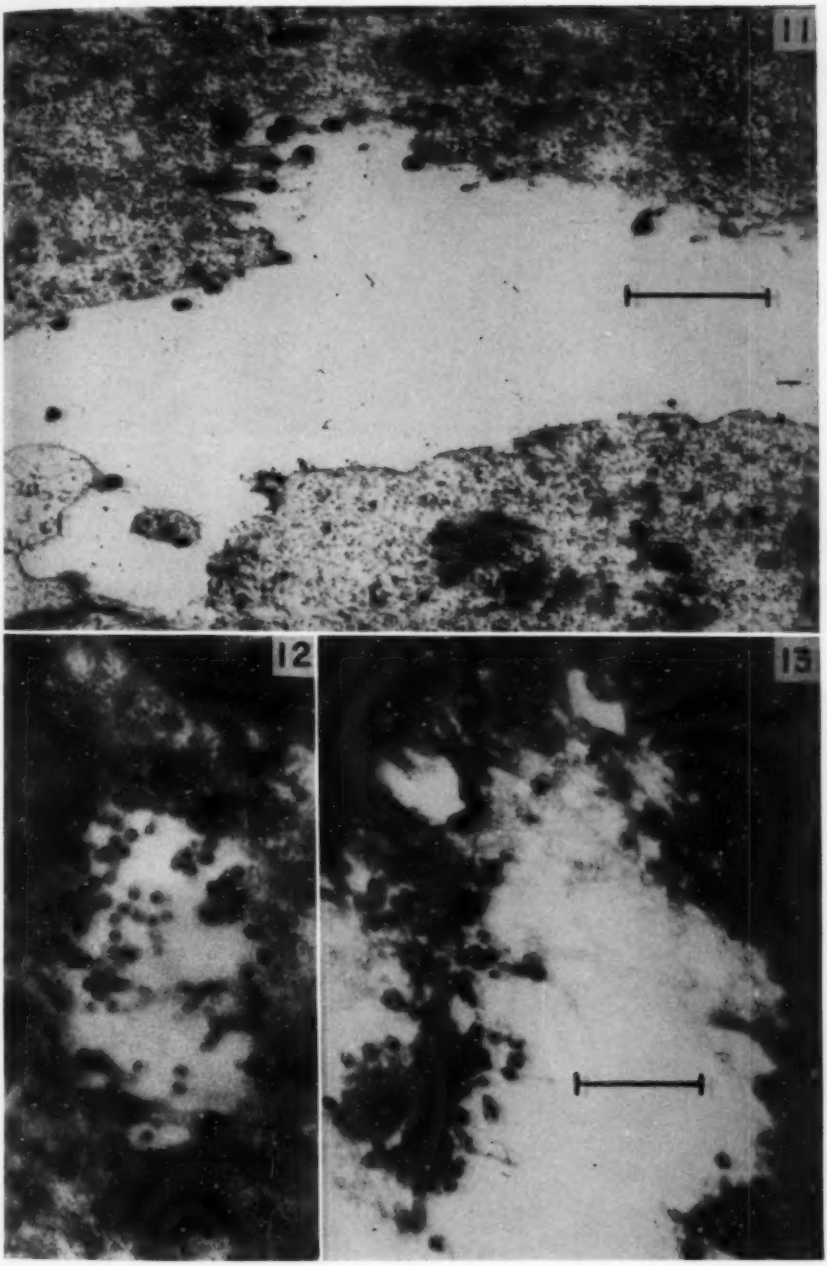
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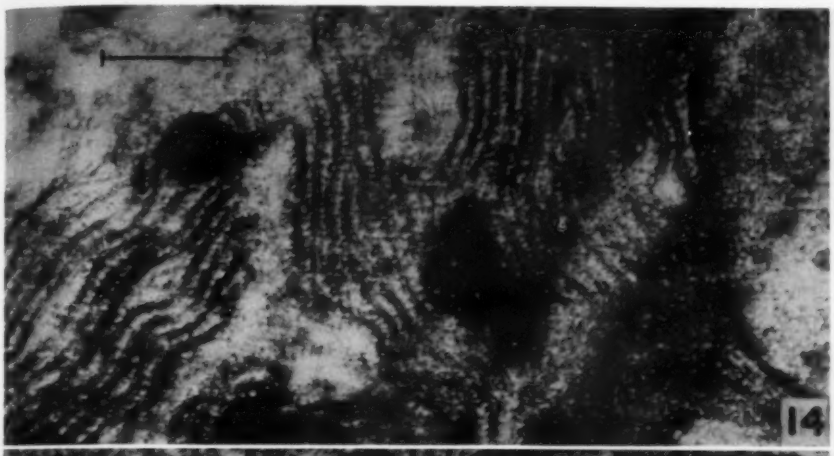
PLATE VI

FIG. 14 Section 241. Section of heavy endoplasmic reticulum of inciter free tumor. This type of reticulum common in other types of glandular cells was not common in the spontaneous mammary tumors. × 22,300.

FIG. 15. Section 216. Unusual heavily coiled nucleolus. This was common in this particular tumor, but was not usually present in spontaneous mammary tumors. × 14,000.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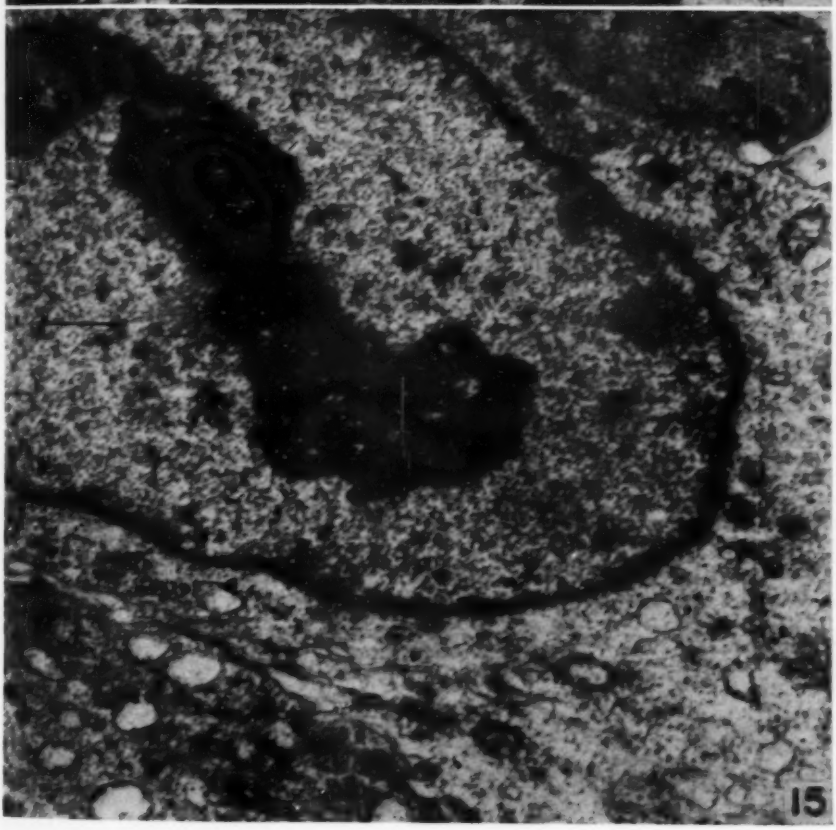
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PLATE VII

FIG. 16. Section 639. Elongated tumor cell invading stroma. Endoplasmic reticulum all through the extended cytoplasm. Knob-like extensions from the cell surface were particularly common in this tumor. × 10,000.

16

FUSIFORM ANEURYSM OF THE ASCENDING AORTA ASSOCIATED WITH MEDIONECROSIS

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Fusiform aneurysm of the ascending aorta due to cystic medionecrosis, unassociated with Marfan's syndrome, has been described infrequently. We have been unable to find any recorded instance in which this diagnosis was correctly made antemortem. This report describes a patient in whom the proper clinical diagnosis was made and surgical treatment undertaken. Because a review of the pertinent literature has revealed striking similarities in the clinical features of previously reported cases, an attempt has been made to summarize these. Recognition of this disease has assumed practical importance in view of the evidence that fusiform aneurysm of the ascending aorta due to medionecrosis is amenable to surgical treatment.

Case report.—JHH 293069. S. L., a 39-year old Negro, was admitted to the Johns Hopkins Hospital in March, 1955, for evaluation and treatment of congestive heart failure and a fusiform aneurysm of the ascending aorta. There was no family history suggestive of Marfan's syndrome. The patient was hospitalized 11 years before, at age 28, because of meningococcemia, and 9 years before with pneumococcal pneumonia. During those hospitalizations his blood pressure was normal (115/65), there were no cardiac murmurs, serological tests for syphilis were negative, and chest X-ray showed a normal heart and aorta. Recovery from both infections was uneventful. At age 32, a soft aortic diastolic murmur was heard for the first time, during a routine physical examination at his place of employment. Serological tests for syphilis again were negative and the blood pressure was normal. Chest X-ray showed slight cardiomegaly (Fig. 1). At age 35 an aortic systolic murmur was also heard, and the blood pressure was 140/60. Chest X-ray showed dilatation of the ascending aorta (Fig. 2). The patient developed exertional dyspnea at age 36, and at age 38 he was hospitalized because of severe dyspnea at rest associated with an episode of poorly described precordial pain. The blood pressure was 128/55–40. The heart was enlarged to the left anterior axillary line, and a diastolic thrill was palpable in the second and third right intercostal spaces. Aortic systolic and diastolic murmurs were heard, and a gallop rhythm was present. Serological tests for syphilis, including a *Treponema* immobilization test, were negative. Electrocardiogram showed left axis deviation and abnormal T waves. Roentgenographic studies revealed a pulsatile fusiform aneurysm of the ascending aorta, and left ventricular enlargement (Fig. 3). The patient's

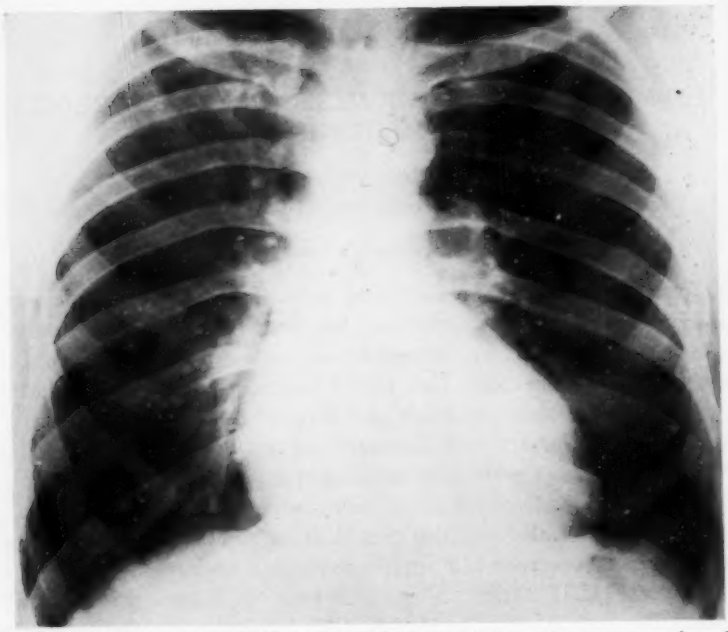

FIG. 1. Chest X-ray, 11–13–47, showing left ventricular enlargement and probable dilatation of ascending aorta.

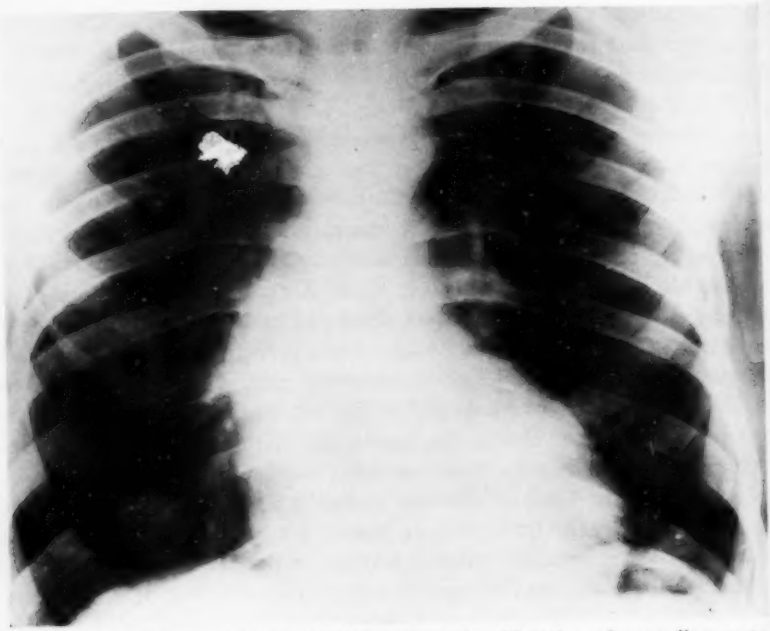

FIG. 2. Chest X-ray, 6–29–51, showing progressive dilatation of ascending aorta

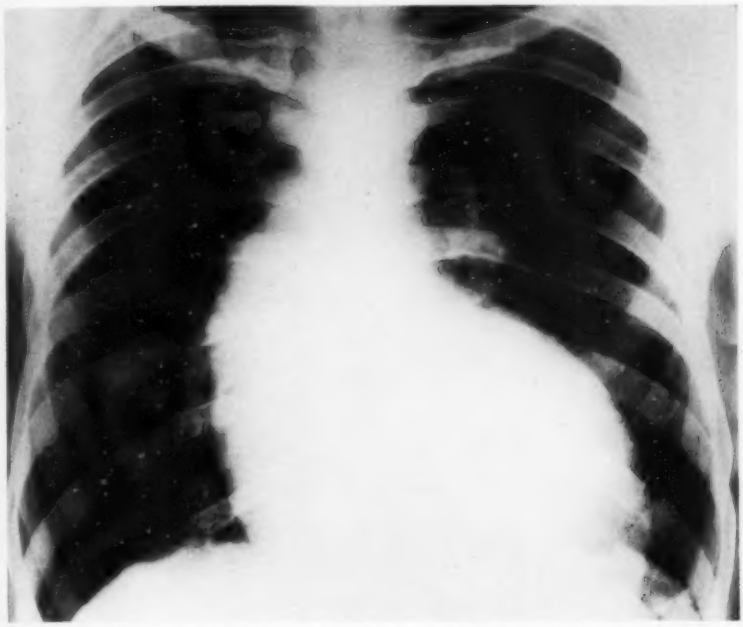

FIG. 3. Chest X-ray, 4–29–54, showing further dilatation of ascending aorta and increasing cardiomegaly.

symptoms of exertional dyspnea and orthopnea were never completely relieved despite intensive treatment with low sodium diet, digitalis and diuretics, and one month before his final admission, hospitalization was again necessary for severe heart failure and anginal pain. The blood pressure was 140/30. There were no stigmata of Marfan's syndrome. The apex impulse was diffuse in the midaxillary line, where a loud, harsh systolic murmur, transmitted into the axilla, could be heard. A loud systolic murmur was heard in the second right intercostal space and was transmitted into the neck. A high-pitched decrescendo diastolic murmur, with an associated thrill, was audible in the third and fourth intercostal spaces on the right. Serologic tests for syphilis, including a *Treponema* immobilization test, again were negative. A diagnosis of aortic aneurysm due to medionecrosis was made. The patient responded poorly to a medical regimen for congestive heart failure and remained critically and severely ill. He was readmitted one month later for surgical treatment of the aneurysm and the associated aortic valve regurgitation.

Left anterior thoracotomy was performed on March 19, 1955 by Dr. Henry Bahnson. It was found that the diastolic thrill disappeared after manual compression of the fusiform aneurysm. The aneurysm was about 20 cm. in diameter, extending from the base of the heart, up to the origin of the in-

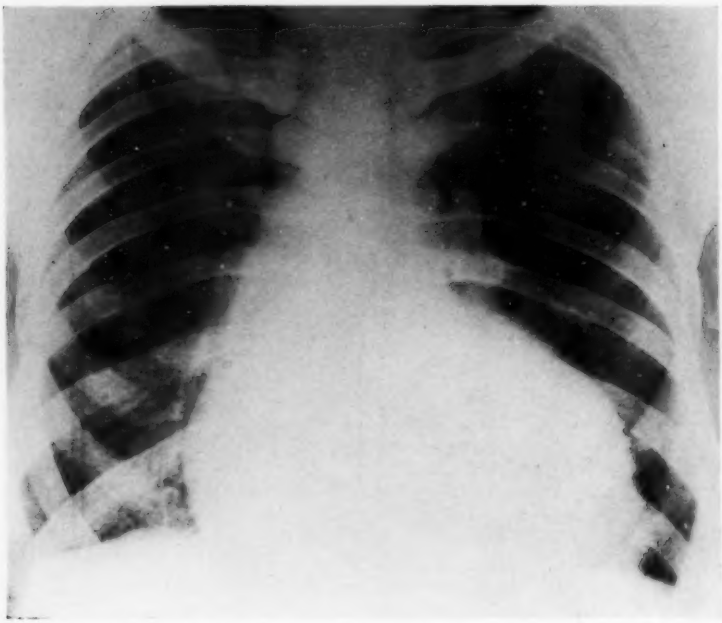

FIG. 4. Chest X-ray, 5–26–55, five weeks after operation.

nominate artery where it ended abruptly. The dilated portion was surprisingly free from adventitial scarring and adhesions. Partial resection and plication of the aneurysmal wall was carried out, with the result that the caliber of the remaining aorta was restored to normal. The aorta was then reinforced with a nylon scultetus binder.

The patient's postoperative course was uneventful and his blood pressure stabilized at 140/90. The previously described diastolic murmur along the right sternal border could not be heard, although a soft blowing diastolic murmur appeared 2 months later (Fig. 4). A soft aortic systolic murmur persisted postoperatively. The patient's improvement was striking. He resumed light work and was asymptomatic until 5 months after operation, when he died suddenly.

*Autopsy**.—The repaired aorta was intact. The heart weighed 900 gm. due almost entirely to hypertrophy of the left ventricle. The aortic valve cusps were normal. The main coronary arteries were patent although a small, hyalinized, subendocardial infarction of the left ventricle was found. Sections

* Appreciation is expressed to the Baltimore City Medical Examiners for providing this information and tissue specimens.

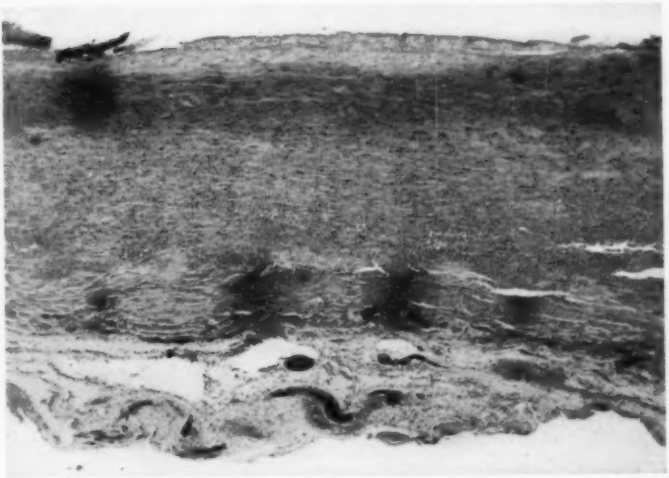

FIG. 5. Microscopic section of aneurysmal wall. H & E stain × 40.

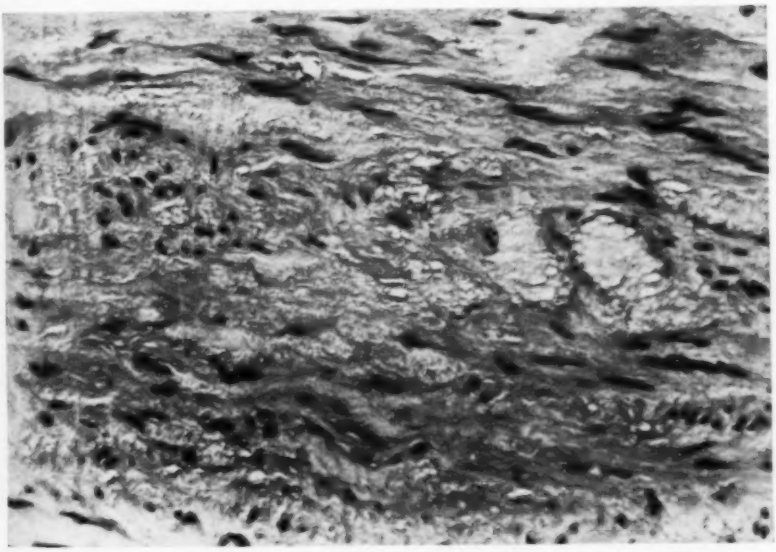

FIG. 6. Microscopic section of aneurysmal wall. H & E stain × 500.

of the ascending aorta obtained at operation and postmortem showed characteristic changes of *idiopathic cystic medionecrosis* (Figs.5 and 6).

Summary.—This patient had a progressively enlarging fusiform aneurysm of the ascending aorta over an 8-year period. Symptoms of chronic congestive

heart failure developed 4 years after an aortic diastolic murmur appeared. There was nothing in the patient's history to suggest even remotely that he had syphilis, acute rheumatism, or congenital heart disease. It was inconceivable that the patient could have had bacterial endocarditis of the aortic valve to account for his diseased aorta and aortic valve insufficiency. By exclusion, the diagnosis of cystic medionecrosis of the aorta was left as the only reasonable possibility. The patient had had episodes of substernal pain and on the basis of clinical findings it was not possible to exclude completely a dissecting aneurysm. However, substernal pain and other symptoms of cardiac disease did not appear until 4 years after the diastolic murmur and aneurysm were first detected. His response to surgical treatment was gratifying. The cause of death was not determined with finality, but he may have had a cerebrovascular accident or ventricular fibrillation. (The brain was not examined at postmortem.)

DISCUSSION

Idiopathic cystic medionecrosis of the aorta, first described by Gsell (1) and Erdheim (2, 3), has been incriminated in a variety of abnormalities of the aorta. The clinical syndromes associated with this type of medial change are: (1) dissecting aneurysm of the aorta, in which the dissection is chronic and frequently produces manifestations of aortic valve incompetency (15, 16); (2) dissection or rupture of the aorta resulting in sudden death (4, 5); (3) dissecting and fusiform aneurysms associated with familial arachnodactyly, iridodonesis, and congenital cardiovascular abnormalities, i.e. Marfan's syndrome (6, 7, 18); (4) fusiform aneurysm of the ascending aorta unassociated with Marfan's syndrome (8, 9, 10, 11, 12). Medionecrosis is, of course, often found at autopsy, unassociated with changes in the integrity or function of the aorta (13, 14).

We have been able to collect from the literature only 5 well documented cases of fusiform aneurysm of the aorta due to medionecrosis. Although this might indicate the rarity of the condition, during the past 6 months we have observed 3 additional patients with fusiform aneurysm proven at operation to be due to medionecrosis. Syphilitic aortic aneurysms are being seen less frequently and there is every reason to believe that medionecrosis will become one of the commonest causes of aneurysm of the ascending aorta in the future.

There are striking clinical and pathological similarities between the reported cases of fusiform aneurysm of the aorta due to medionecrosis and the case described here. In none of these patients were there stigmata of Marfan's syndrome. *Age.*—Five of the six patients were under the age of 50, and two under the age of 40. *Sex.*—Five of the six patients were males. *Heart failure.*—Severe congestive heart failure was the most prominent symptom in all of the cases, including the one reported here. *Cardiac murmurs.*—In every patient

there were significant murmurs in the aortic valve region, but in only one instance (bicuspid valve) was significant abnormality of the valve leaflets found at postmortem examination. In five patients an aortic diastolic murmur was heard, and an aortic systolic murmur was present in four patients. The diastolic murmur, and in some cases the accompanying thrill, was impressive even in the absence of peripheral signs of aortic regurgitation. Transmission of the aortic diastolic murmur was down either the right or left sternal border. Machinery-like murmurs have been described (10). *Blood pressure.*—Significant hypertension was observed in only two patients. Occasionally, widening of the pulse pressure was found. *Chest pain.*—Precordial pain was a prominent feature in some instances, even though unassociated with aortic dissection. The patient presented here had pain suggestive of coronary artery disease. A subendocardial myocardial infarction was found at postmortem examination, although the main coronary arteries were patent. Angina-like pain may be produced in these patients by narrowing of the coronary ostia because of the aneurysmal dilatation and diseased aortic media. Dissection of the aorta with severe pain can also occur in these patients, and in two cases seen recently aortic dissection had apparently occurred prior to operation. *Duration of illness.*—In the reported cases, death occurred 6 months to 11 years after onset of symptoms. *Cause of death.*—Severe congestive heart failure was the cause of death in all of the cases except the one reported here.

Rottino (14) has pointed out that cystic medionecrosis occurs predominantly in the ascending aorta. In the five previously reported cases, and in the one described here, fusiform aneurysm of the aorta due to medionecrosis involved the ascending aorta proximal to the orifice of the innominate artery. The gross appearance of the dilated vessel differed from that seen with syphilitic aneurysm, there being no adventitial scarring, with the result that the aorta was easily freed from contiguous tissue.

The prognosis is poor in patients with fusiform aneurysm due to medionecrosis when symptoms of congestive heart failure develop. Because aneurysmorrhaphy is feasible, and was easily accomplished in the present case, the establishment of the diagnosis assumes practical importance. If death does not occur from congestive heart failure, there is the ever present hazard of dissection of the aorta, and wrapping the ascending aorta with nylon or cellophane may prevent this complication.

The diagnosis of fusiform aneurysm of the aorta due to medionecrosis is not difficult if syphilis can be excluded. Negative serological tests for syphilis, including the *Treponema* immobilization test, without a history of treatment for syphilis, would be strong evidence against the diagnosis of luetic aortitis. Differential diagnosis includes subaortic stenosis with poststenotic dilatation of the aorta, aortic insufficiency due to rheumatic heart disease, syphilis or healed bacterial endocarditis. However, these conditions can usually be

excluded by an appropriate history, the presence of aortic stenosis without insufficiency, or the finding of peripheral signs more indicative of primary aortic valve disease. Elimination of healed or chronic dissection of the aorta is more difficult, but in such patients dilatation of the aorta tends to occur abruptly, and hypertension is usually prominent. Often, a history of pericardial friction rub, severe episode of chest pain, or an acute attack of vascular collapse will be obtained if dissection has occurred. Even this disorder, however, is sometimes benefited by surgical therapy (17).

Fusiform aneurysm of the aorta due to medionecrosis, as would be expected, occurs occasionally in patients with Marfan's syndrome, and if the stigmata of this disorder are present the clinical diagnosis of medionecrosis is, of course, facilitated.

The correct clinical diagnosis of fusiform aneurysm due to medionecrosis was possible in the case reported here by the correlation of information gathered over a period of 12 years. Significant was the progressive dilatation of the ascending aorta and development of an aortic diastolic murmur; murmurs appeared prior to the onset of chest pain; the aneurysm was confined to the ascending aorta; there were few peripheral signs of aortic valve incompetency early in the course; evidence for syphilis, rheumatic heart disease and bacterial endocarditis were completely absent.

SUMMARY

A case is presented describing the occurrence of fusiform aneurysm of the ascending aorta, unassociated with Marfan's syndrome, caused by cystic medionecrosis. The clinical features presented by this patient and others described in the literature are discussed. As far as can be determined the patient reported here is the first in whom the diagnosis was established antemortem. Treatment of the disease is discussed, indicating that such aneurysms of the aorta are amenable to surgical therapy.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

January 9, 1956

THE EFFECT OF HYDROCORTISONE UPON THE COURSE OF PNEUMOCOCCAL PNEUMONIA TREATED WITH PENICILLIN

HENRY N. WAGNER, JR., IVAN L. BENNETT, JR., LOUIS LASAGNA, LEIGHTON E. CLUFF, MIRIAM B. ROSENTHAL AND GEORGE S. MIRICK

(Presented by Dr. Bennett)

This paper appeared in the March, 1956 issue of the Bulletin.

STUDIES ON THE ANTI-INFLAMMATORY EFFECTS OF CORTISONE

W. BARRY WOOD, JR.

The results of studies on the pathogenesis of acute inflammation, carried out in collaboration with Dr. Fred Allison, Jr., and Mary Ruth Smith, were presented with the aid of a short motion picture film. The findings have recently been published in detail (J. Exper. Med., 1955, **102:** 655–676) and summarized as follows:

I. The Inflammatory Reaction to Thermal Injury as Observed in the Rabbit Ear Chamber.

A special adaptation of the rabbit ear chamber has been devised to study *in vivo*, under high magnification, the acute inflammatory reaction to thermal injury. Systematic observations of the cellular response have led to the following conclusions.

1. Contrary to the commonly accepted view, vasodilatation does not always precede the adherence of leucocytes to vascular endothelium.

2. The fact that leucocytes often adhere to one another as well as to the endothelium indicates that the increased adhesiveness characteristic of the early stages of inflammation is not limited to the surfaces of the endothelial cells.

3. The sharing of erythrocytes and platelets in this increased stickiness suggests that a "plasma factor" is involved. There is indirect but as yet inconclusive evidence that the plasma factor may concern the clotting mechanism of the blood.

4. The adherence of leucocytes to the endothelium is usually first noted on the side of the vessel closest to the site of injury. This previously undescribed phenomenon of "unilateral sticking" is in keeping with the concept that the vascular reaction is caused by products of cellular damage which diffuse to the vessel from the site of injury.

5. Leucocytes always become adherent to the endothelium before penetrating the vessel wall. They often migrate about for some time on the endothelial surface before undergoing diapedesis.

6. Although no definite stomata are at any time visible in the endothelium, penetrating leucocytes may leave behind temporary defects through which other leucocytes and even erythrocytes may pass.

7. The diapedesis of leucocytes appears to depend primarily upon cellular motility. It may occur in static vessels where there is presumably little if any hydrostatic pressure.

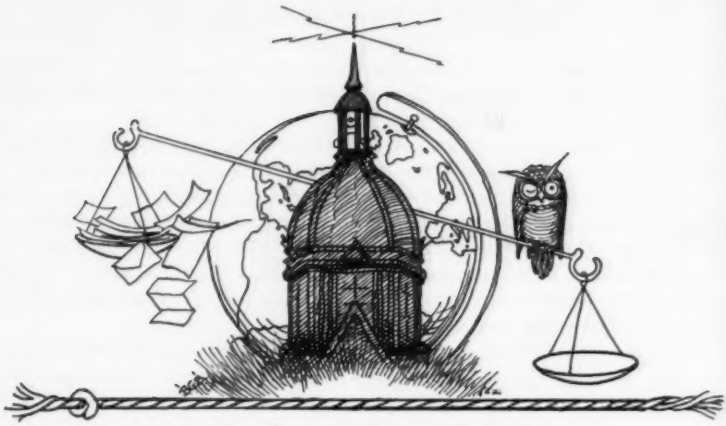
8. The diapedesis of erythrocytes, on the other hand, is a passive process depending upon intravascular pressure. Its occurrence is greatly exaggerated in areas in which intravascular pressure becomes elevated. Such elevations occur as the result of proximal arteriolar dilatation and distal occlusion of vessels.

9. Once they have reached the extravascular tissues the leucocytes move about more or less at random, apparently uninfluenced by any compelling chemotactic force. Their resultant migration, however, is toward the site of injury around which they eventually tend to congregate.

10. The histiocytes normally present in the connective tissue appear to play no role in the type of acute inflammatory reaction produced in these experiments.

II. The Action of Cortisone on the Inflammatory Response to Thermal Injury.

The anti-inflammatory action of cortisone upon the acute cellular response to thermal injury has been systematically studied in the rabbit ear chamber. The hormone has been shown to suppress the reaction of acute inflammation in its earliest recognizable phase; i.e., that involving vasodilatation and the adherence of leucocytes to the vascular endothelium. Evidence has been presented that the anti-inflammatory effect of the hormone cannot be explained on the basis of its vasoconstrictive properties alone. The experimental observations support the hypothesis that cortisone exerts a direct protective action upon endothelial cells and leucocytes, and that in so doing, it renders them refractory to the tissue products which initiate inflammation.



BOOK REVIEWS

Benjamin Franklin. Some Account of the Pennsylvania Hospital. Printed in Facsimile with an Introduction by I. BERNARD COHEN. 40 pp., $3.25. The Johns Hopkins Press, Baltimore, Maryland.

Reproduced here in facsimile is a book written and published over 200 years ago by one of the greatest Americans of all times. This year, the 250th anniversary of Franklin's birth, there is renewed interest in his personality and contributions.

Franklin was in his own words a "doer of good", a role in life perhaps now held in something less than the highest regard but in Franklin's estimation the loftiest of man's worldly pursuits. In his introduction Cohen traces the development of Franklin's strong social conscience. Germinating in Calvinistic Boston, it developed to full blossom in the warm sun of Quaker Philadelphia. It will surprise many to discover the strong influence, in matters both philosophical and pragmatic, which Cotton Mather, usually thought of as the epitomy of Puritan bigotry, had on the liberal Franklin. According to the analysis of Cohen the relationship between the thinking of the two men was impressively close. The contributions of Mather to science and medicine have only of relatively recent years been appreciated; vicarious contributions through his influence on Franklin are also noteworthy.

In the second place, Franklin was a master of the fine art of promotion. He had the following to say about how to raise funds: "I advise you to apply to all those whom you know will give something; next, to those whom you are uncertain whether they will give anything or not, and show them the list of those who have given; and, lastly, do not neglect those who you are sure will give nothing, for in some of them you may be mistaken". Everyone is aware that his promoting resulted in a university, a fire company, a regular constabulary, a circulating library and many other institutions in addition to the one directly concerned in this discussion. There is a modern ring to the entire book, right through to the last page which bears a form for use by the solicited in making his contribution.

Part of the pleasure in reading and possessing this book resides in the fine example of Franklin's printing it is. The title page is oustanding. In the body of the book the print is a bit small and the pages cramped but in general the effect is pleasing.

The arguments for hospitals as advanced by Franklin were as follows: (1) Banded together men (not only physicians but all who in any capacity directly or indirectly minister to the sick) can do more for the commonweal than can be done if each works independently. (2) The hospital can restore sick individuals to a useful place in society. (3) The hospital is a

proving ground for young physicians. The quality of medicine in the community is elevated by the existence of the hospital. (4) The hospital gives "to the beggar in America a degree of comfort and chance for recovery equal to that of a European prince in his palace". (5) "The difference between nursing and curing the sick in a hospital, and separately in private lodgings, with regard to the expense, is at least as ten to one".

One reads with interest the admission policies (e.g., " . . . no patients shall be admitted whose cases . . . do not require the particular conveniences of an hospital") and the provisions for the keeping of records ("the practitioners shall keep a fair account, in a book provided for that purpose, of the several patients under their care, of the disorders they labour under, and shall enter in the said book the recipes or perscriptions they make for each of them"). The statistics on the 117 patients admitted between February, 1752, and April, 1754 are given. The diagnosis and the outcome are given. Some of the diagnoses are unfamiliar; most are non-specific and too general for modern tastes. For example, there was one case of "mortification"; he died. "Gutta serena" was cured in one patient. Lunatics were represented by 18 of the 117; of the 18, two were cured, three "relieved" (?), and six taken away by their friends. In fact, in the introduction, Franklin states, as one of the purposes of institution, care of those "who unhappily became disordered in their senses, wandered about, to the terror of their neighbors".

This book is an important document in the history of medicine; it is interesting reading; it may hold practical hints for those concerned in "drives" for charitable causes.

VICTOR A. MCKUSICK

Handbook of Toxicology, Vol. I. Acute Toxicities of Solids, Liquids and Gases to Laboratory Animals. Edited by WILLIAM S. SPECTOR. Prepared under the direction of the Committee on Handbook of Biological Data Division of Biology and Agriculture; The National Academy of Sciences; The National Research Council. 408 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa.

This volume presents data collected from the literature on the acute toxicity of various substances for various species of animals—mostly mice, rats, and rabbits. There are two tables and an index. Table I contains data on the fatal doses of 2120 compounds determined by oral or parenteral administration; Table II, data on lethal concentrations of gases and vapors in the respired air of 243 substances. A literature reference is given for each item. The book should prove of value to those wishing a first approximation of the toxicity of a chemical and a literature reference.

E. K. MARSHALL, JR.

Clinical Neurosurgery: Proceedings of the Congress of Neurological Surgeons. Vol. 2. 1954. Edited by RAYMOND K. THOMPSON AND IRA J. JACKSON. 173 pp., $6.75. The Williams & Wilkins Co., Baltimore, Md.

This is the second small volume published by this group of neurological surgeons to report the transactions of its meetings. Conforming with previous custom, several major papers were presented by the guest of honor. There are also reports of panel discussions on various aspects of trauma to the head and neck.

To the reviewer and to many others in the fields of neurological medicine and surgery, it is a delight to have avilable this tribute to Dr. Kenneth McKenzie. Through his personal characteristics, his masterful practice of clinical neurosurgery, and his inspiring teaching, he has had a strong influence on the development of these fields and the men in them. The results

of his experience, and his several important original contributions, have unfortunately been rather scattered in the literature. It is a welcome addition to have assembled in one volume his discussions of the gliomas, eighth nerve tumors, spasmodic torticollis, Ménière's syndrome and trigeminal neuralgia.

The two additional panel discussions contain little new but offer interesting consideration of several features of these vexing problems.

J. W. M.

Henry Ford Hospital International Symposium on Cardiovascular Surgery. Studies in Physiology, Diagnosis and Techniques. Edited by CONRAD R. LAM. 543 pp., $12.75. W. B. Saunders Co., Philadelphia, Pa.

This symposium is beautifully engineered. The publication of symposia, like the planning and execution of same, seems to be a difficult undertaking, if one can judge by the products, excellent, mediocre, and poor, that one sees. I marvel that the editor and pilot of the entire undertaking, Conrad Lam, thoracic surgeon at the Henry Ford Hospital, was able to get such a profusely illustrated and accurately reproduced collection of papers—with discussions—off the presses so soon after the symposium. Often there is a delay of over a year with attendant decrease in the usefulness of the publication.

The cardiologist owes much to the surgeon. Sir Russell Brock does not hesitate in "rubbing in" that point in the foreword. Cardiology has made great strides in the last decade, some in non-surgical areas, due to a certain stimulus provided by the surgeon. One has merely to compare the contents of previous volumes of the American Heart Journal or British Heart Journal with more recent volumes to be impressed with the change. The surgeon has insisted on diagnoses accurate to the last anatomical detail; he has stimulated physiological study and physiological thinking about cardiological problems. "Many time-worn beliefs and shibboleths have had to be discarded," Brock states, in his introduction to a quite comparable collection of papers on Surgery of the Heart and Thoracic Blood Vessels in the British Medical Bulletin (1955).

Certainly no one would question the tremendous impetus provided by the fresh and vital approach of the surgeon. However, Sir Russell exaggerates unrealistically in such statements as that "cardiac surgery has been responsible for a much-needed renaissance in cardiology". He sells short the importance of the patient, laborious observations of physicians, which guided the launching of these dramatic expeditions into the rarefied atmosphere of cardiovascular surgery and without which they would have been, if not impossible, tardier and less successful.

No attempt will be made to review the contents of this sizable volume. The fact that there were 60 participants and 17 discussants will give some indication of the scope of the symposium. In fact all conceivable aspects of cardiovascular surgery are touched upon. Suffice it to say, this book is probably at the moment the definitive treatise on the subject.

A compliment is due Elton Hoff, a former student of the Johns Hopkins School of Art as Applied to Medicine, for his fine drawing of the heart in mitral stenosis which serves as the frontispiece, in color, of the volume.

VICTOR A. MCKUSICK

The Pharmacological Basis of Therapeutics, 2nd edition. By LOUIS S. GOODMAN AND ALFRED GILMAN. 1831 pp. $17.50 The Macmillan Co., New York, N. Y.

The first edition of Goodman and Gilman ushered in an era of unprecedented advance in the field of drug therapy; the second edition comes as a welcome review of these developments. Keeping pace are new chapters and completely revised sections on "Drugs Effective

in Convulsive Disorders", "Natural and Synthetic Narcotics", "Autonomic Blocking Agents", "Histamine and Antihistaminic Agents", "Drugs Used in the Chemotherapy of Tuberculosis and Leprosy", "Antibiotics", "Hormones and Hormone Antagonists". Throughout, there is thorough and critical review of the pharmacological literature with balanced assessment of conflicting views and full citation of carefully selected source material.

This is a volume that has served admirably as a textbook in the pharmacology course, and it will continue to serve during clinical and post-graduate years as a reference. The length of this text is a small price to pay for having available a volume so complete in its coverage of the subject. The excellent organization with prominent section headings should facilitate the selection of material for emphasis and study. Particularly effective are the illustrative laboratory records and clinical charts, each supplied with a complete protocol of the experiment.

One might ask for a little more emphasis on some of the more generalized aspects of pharmacology. For example, a more extensive comparative and orienting discussion would be helpful in the section on "Absorption, Distribution, Fate, and Excretion of Drugs" even though such material is now adequately presented under the individual drugs.

This second edition of "The Pharmacological Basis of Therapeutics" is so thorough a revision that it stands as a new contribution and a worthy successor to the classic first edition.

MORRIS ROSENFELD

Michael Servetus. A Translation of his Geographical, Medical, and Astrological Writings with Introductions and Notes. By CHARLES DONALD O'MALLEY. 208 pp., $3.00. The American Philosophical Society, Philadelphia, Pa.

This monograph is of interest to the medical historian. It is the first English translation of his lesser known writings on medicine, geography and astrology. Whilst Servetus is well known as a theologian and as one among the first group of protestants against the Roman Church in the 15th and 16th centuries, little is known of him as a scientist. This monograph attempts to reveal this side of him in presenting an English translation of his writings.

Although his discourses on Galenic medicine, and his defense of Hippocrates and Galen are scientifically not great, they reveal the intensity and zeal of this great reformer.

The translator has done a good translation and has not fallen into the trap of interpreting and rewriting prose for us, a trap into which so many translators fall.

The book is recommended for its historical interest.

JOHN COLLINS HARVEY

BOOKS RECEIVED FOR REVIEW

Arthroplasty. By ST. J. D. BUXTON. 126 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa.

Biochemistry for Medical Students, 6th edition. By W. V. THROPE. 542 pp., $6.75. J. B. Lippincott Co., Philadelphia, Pa.

Cybernetics. Transactions of the Tenth Conference, April 22, 23 and 24, 1953. Edited by HEINZ VON FOERSTER. 100 pp., $2.75. The Josiah Macy, Jr. Foundation, New York, N. Y.

The Interpretation of the Unipolar Electrocardiogram. By GORDON B. MYERS. 164 pp., $4.75. The C. V. Mosby Co., St. Louis, Missouri.

Lehrbuch der Verdaunungskrankheiten. By NORBERT HENNING AND W. BAUMANN. 562 pp. Ganzleinen DM 66. ($18.35). Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Medical Book Corp., New York, N. Y.

James Parkinson 1755–1824. Edited by MACDONALD CRITCHLEY. 268 pp., $4.50. St. Martin's Press, Inc., New York, N. Y.

Pathologic Physiology. Mechanisms of Disease, 2nd edition. Edited by WILLIAM A. SODEMAN. 963 pp., $13.00. W. B. Saunders Co., Philadelphia, Pa.

Physiotherapy in Some Surgical Conditions. By JOAN E. CASH with a foreword by F. A. R. STAMMERS. 350 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

Planning New Institutional Facilities for Long Term Care. By EDNA NICHOLSON. Forewords by LEONARD A. SCHEELE, EDWIN L. CROSBY, G. WARFIELD HOBBS AND EDWIN B. MORRIS, JR. 358 pp., $4.50. G. P. Putnam's Sons, New York, N. Y.

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